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ANATOMY OF THE AXIS OF THE BANANA[1]

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(WITH TEN FIGURES)

Introduction

Although the development and anatomy of the inflorescence, the root, and the leaf of the banana have recently been the subject of studies by WHITE (**14**), ACQUARONE (**1**), and the writer (**10, 11, 12**), no detailed investigation appears ever to have been made of the anatomy of the axis. Numerous incidental references to the rhizome of the banana may be found in the literature, however, to the more important of which allusion will be made in due course. In the present paper the term "axis" is employed in its widest sense, to include the rhizome or bulb, the aerial stem, and the rhachis of the inflorescence. This study was begun late in 1928 at the Research Station of the United Fruit Company near Almirante, Panama, where only a preliminary survey of the subject was attempted, and was completed during 1930 at the Lancetilla Experiment Station of the Tela Railroad Company, near Tela, Honduras. In Panama a seeded banana known as "Martini," a variety of *Musa sapientum* sub. *seminifera*, was used; while in Honduras, since this type was not available, I employed the well known Gros Michel variety, the chief banana of commerce. No important anatomical difference between the bulbs of the two varieties was observed.

[1] Botanical contribution from the Johns Hopkins University no. 115. The work reported in this paper was begun while the writer held a Johnston Scholarship of this University, and completed under a Fellowship of the National Research Council of Washington.

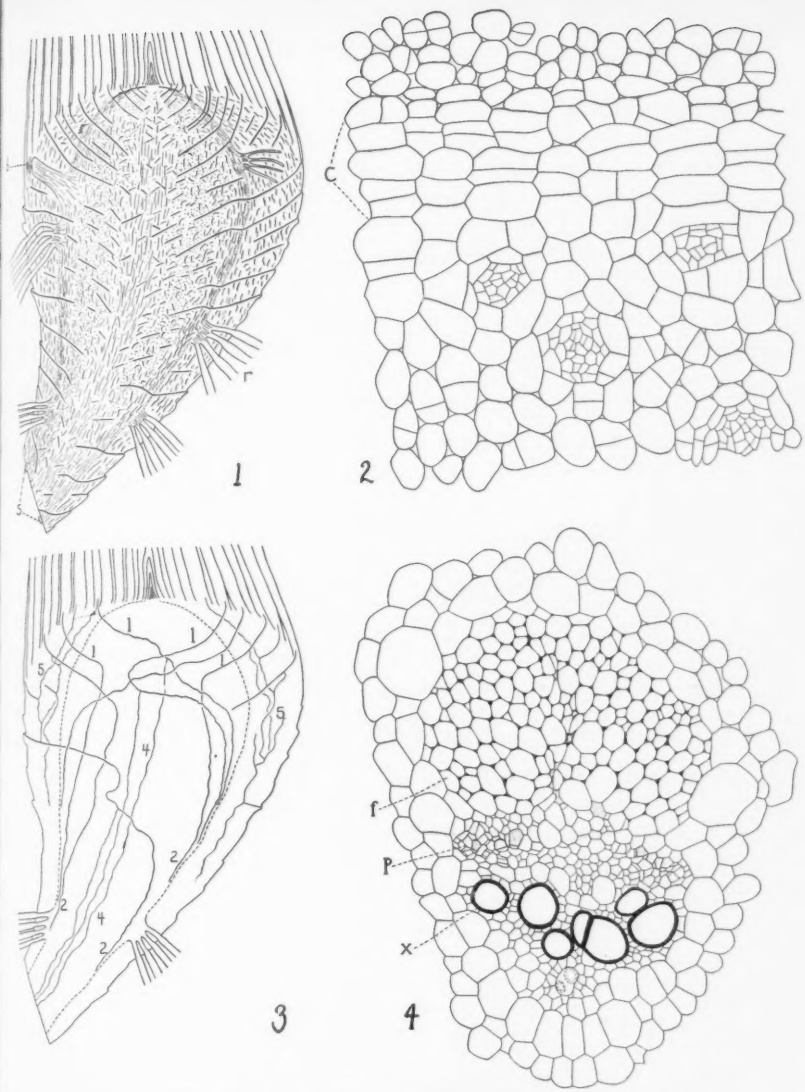
General features

The banana plant has a thick, many branched rhizome, each short branch of which expands rapidly toward its apex and is known by planters as a "bulb." From the upper surface of the bulb arises a rosette of leaves with elongated leaf sheaths closely overlapping to form a tall false-stem. The sides and older portions of the bulb are covered with the close-set circular scars left by the detachment of former leaves. Ultimately the axis elongates, pushes up through the center of the false-stem, and emerges from its upper extremity to bear the flowers and fruit. As in other herbaceous plants, the life of this particular shoot ends with its production of fruit, but the bulb at its base sends forth branches which develop into new flowering shoots.

The lateral buds of the bulb are rather unusual in their position. They are situated, not in the axils of the leaves, but on the side of the stem opposite these, between the two free margins of the leaf sheath (**10**, figs. 1, 2). This circumstance suggests that the rhizome of the banana is in reality a sympodium; that the apparently lateral bud is in reality a terminal bud of arrested development which has been pushed to one side by the development of the true lateral bud, originally situated between it and the axil of the leaf. Although this view of the situation would bring the banana into morphological conformity with the majority of plants, there appears to be nothing beyond the peculiar position of the buds to support it.

The lateral buds are unable to develop until the leaf sheaths covering them die and decay away. At first they grow horizontally outward and produce large, thick scales representing the leaf sheaths alone. After they reach a certain size, the leaf sheaths become sensitive to gravity and bend upward, erecting the still rudimentary false-stem (SKUTCH **13**). The rhizome more slowly alters the direction of its growth until the growing point faces upward, and increases in thickness until a bulb is formed. In the Gros Michel variety this bulb attains a diameter of more than 30 cm. and a height of 35 cm. or more.

The massive central cylinder is readily distinguished from the thick cortex, in either a longitudinal or transverse section of the bulb, by the plexus of large bundles of generally horizontal course which



FIGS. 1–4.—Fig. 1, median longitudinal section through bulb of large sucker, showing diagrammatically the distribution of vascular bundles. Portions of bundles in plane of section represented by lines, bundles cut transversely by dots; cambium (near apex) indicated by heavy line; very young root rudiments by large black spots: *b*, lateral bud; *r*, lateral root; *s*, secondary zone. ×0.3. Fig. 2, cross-section through cambium (*c*) in region where its derivatives are forming longitudinal bundles, showing four bundles in various stages of differentiation. Camera lucida, ×190. Fig. 3, diagram showing courses of various types of vascular bundles in bulb. Broken line indicates boundary between cortex and central cylinder. The numbers are those under which each type is described in text (cf. fig. 1). ×0.3. Fig. 4, cross-section of leaf-trace bundle just after entering central cylinder of bulb: *f*, fibers; *p*, phloem; *x*, xylem. ×76.

occupies the outer region of the former (fig. 1). Just within the horizontal bundles is a narrow zone where all the bundles, although flexuous, run in a generally longitudinal direction. Both longitudinal and horizontal bundles are distinguished from all others found anywhere in the axis or leaves by their amphiphloic structure. Since they arise from a cambium-like meristem, they are designated as "secondary bundles." The bundles in the primary portion of the central cylinder present a bewildering disarray, especially in longitudinal section; for it is only the upper portions of the leaf-trace bundles which follow a straight course for any considerable distance, and short lengths of the other bundles are encountered running in every direction apparently at random. In a cross-section of the bulb, the secondary longitudinal bundles may be distinguished from the primary bundles of the central cylinder by their more even spacing and the absence of bundles with a tangential or oblique course in the region they occupy. They are, of course, interrupted by the radially directed leaf-trace bundles entering from the cortex.

The adventive roots arise, as in the rhizomes of other monocotyledons, from the surface of the central cylinder, almost invariably in longitudinal groups of four.

Rhizome

Origin of secondary bundles and adventive roots

While the collateral primary bundles differentiate directly from the massive tissues produced by the apical meristem, the amphiphloic bundles of the secondary zone arise from a special meristem. A cambium-like layer is of wide-spread occurrence just behind the apex of the rhizomes of a number of monocotyledons, and is associated with the production of adventive roots. Mangin (4) recognized this special meristem, which gives rise, not only to the adventive roots but also to a plexus of bundles which centers about the base of each root, or each group of them where they arise in a series, and serves to connect them with the leaf-trace bundles. For this reason he designated the meristem as the "couche dictyogène," and the plexus of bundles to which it gives rise as the "réseau radicifère." Unlike the more enduring cambium of such arborescent monocotyledons as *Dracaena* and *Yucca*, it is associated only with the produc-

tion of the roots, and does not occur on portions of the stem or rhizome where roots are not formed. Thus in the rhizome of *Iris florentina*, which creeps along the surface of the ground and bears roots on its lower side only, it is only on this side that the "réseau radicifère" appears. Later PETERSEN (**5**) described the same phenomenon, and discovered the fugacious cambium near the apex of the rhizome in a number of families of monocotyledons which he examined, with the exception of the Orchidaceae. He concluded that, "There exists among the monocotyledons a series of transitions from stems in which there is no localized meristem—here we do not refer to the growing point—to those where there arises a secondary meristem producing cells in radial series which effects an unlimited growth in thickness." MANGIN had come practically to the same conclusion ten years earlier.

In time of origin the cambium[2] of the banana rhizome differs from that generally encountered in dicotyledonous shoots in not being separated from the apical meristem by a considerable length of stem in which no tangential divisions of the procambial cells take place. In the banana the cambium may already be distinguished at about 5 mm. behind the rounded apex of the bulb, in a region which seems still a part of the growing point, for the surrounding cells are immature and divide rapidly. Thus the cambium is closely related to the primary growing point, and possibly should be considered merely as a special development of it: that here at the outer surface of the central cylinder it remains active longer than elsewhere and assumes a special function. The length of the short cambium cells hardly exceeds their width. The number of tangential divisions which each undergoes is limited; six cells are frequently encountered in a radial series, more rarely seven or eight. Everywhere the cambium is interrupted at frequent intervals by the radial leaf-trace bundles, which

[2] I have doubted the propriety of calling this meristem a cambium, since it differs in so many important respects from the true cambium of gymnosperms and dicotyledons. The bundle-forming meristem of the arborescent Liliiflorae, etc., which has much more in common with the meristem of the banana than with the dicotyledonous meristem, is also designated by this term, which in a loose way is applied to any meristem which undergoes many divisions all in the same plane. Hence for want of a better word it seems best to retain this in the present case. Perhaps a strict terminology would demand a special designation for the type of meristem exemplified in *Dracaena*, *Musa*, etc.

differentiate early. It reaches its greatest activity between 1 and 2 cm. from the apex, where numerous vascular bundles are differentiated from its daughter cells, entirely toward the interior, for it adds nothing to the thickness of the cortex. An entire longitudinal segment of a bundle, including all the various elements of xylem and phloem, is derived from the divisions of a single cell, as in the arborescent Liliiflorae (fig. 2). The first dividing wall may be either tangential or radial in position, apparently more commonly the latter. Thence walls are formed rapidly in all planes, and the small cells which result enlarge and differentiate to form the amphiphloic vascular bundle.

At about 2.5 cm. from the apex the last longitudinal bundle is formed, and horizontal bundles begin to differentiate from the daughter cells of the cambium. These are cut out from a single cell in much the same way as the longitudinal bundles, save that the walls now formed are horizontal instead of vertical. The change from the production of longitudinal to horizontal bundles is abrupt, the plane of division of the mother cells being, so to speak, rotated through 90° without any significant alteration in the shape of the cambium cells. The first rudiments of the horizontal bundles are encountered beneath a root primordium. In the region where horizontal bundles are produced the cambium becomes rather irregular, and at about 3.5 cm. from the apex, where the last bundles begin to differentiate, it dies out altogether. Thus its entire activity extends over an arc hardly more than 3 cm. in length; in the first 2 cm. it produces longitudinal bundles, in the last centimeter, horizontal bundles.

As has been stated, the lateral roots are also initiated by the cambium. At the points where these arise, the cambium becomes very active and divides repeatedly in all planes, forming a common primordium in the shape of a little mound of tissue from which are developed, in acropetal succession, the growing points of the four adventive roots which almost invariably arise together, always in a linear series. Thus there is direct continuity of meristematic tissue from the apical growing point of the bulb to the growing point of the root. MANGIN (4) states that in *Convallaria*, *Sisyrinchium*, *Ruscus*, and *Zea* the body of the root is alone formed from the cambium, while the root cap is produced by the innermost cortical cells. Refer-

ence to his plate 10, fig. 18, of *Ruscus aculeatus*, shows that it is extremely difficult to follow, in the very young root primordium, any clear line of demarcation between cells of cortical and cambial origin. Moreover, as described later, the cambium itself is constantly renewed from the inner cells of the cortex. In the banana it appears that the growing point of the root is derived in its entirety from the cambium. The cortical cells immediately in front of it are soon crushed by its rapid development, and hence further meristematic activity on their part is impossible. The common primordia of the groups of four roots are first clearly visible to the naked eye at about 1.5 cm. behind the apex, and the growing point of the lowest root, the most advanced of the group, is already distinguishable under the microscope at this point.

Since the number of times each cambial cell divides is strictly limited, it is necessary that the cambium be renewed from some source. This is provided by the innermost cells of the cortex, which remain undifferentiated longer than the primary tissues in other regions. As the cambium cells exhaust their possibilities of division, these cortical cells just outside of them begin to divide by tangential walls and take their places in the cambium. At the same time other cells in the cortex differentiate into the innermost cortical bundles. As in the secondary bundles of the central cylinder, each segment of the length of these cortical bundles is derived from a single mother cell.

The cambium in the banana accordingly differs in an important respect from the true cambium of dicotyledons, in which there is an initial cell for each radial row which divides tangentially an indefinite number of times. The former type of meristem is more characteristic of the monocotyledons than a true cambium. RÖSELER (**7**) pointed out that the cambium of the arborescent Liliiflorae divides according to different laws from that of conifers and dicotyledons, and demonstrated the absence of initial cells. SCHOUTE (**8**) found that in the stem of *Cordyline rubra* and other arborescent Liliaceae the cambium during the earlier stages of secondary thickening is of the irregular type just described, for which he proposed the term "Etagencambium" or "storied" cambium; but at a later period the meristematic cells continue to divide indefinitely and so a true initial

cambium arises. LINDINGER (3) states that in the roots of *Dracaena draco* and *D. fragrans* the early storied cambium is also succeeded by an initial cambium as the root becomes older. SCHOUTE (8), and later PHILIPP (6), investigated the origin of cork in monocotyledons and found that in the great majority of cases this is produced almost exclusively by an "Etagencambium," that is, each cork-producing cell exhausts its meristematic potentialities after producing a limited number of cork cells, when its place is taken by another cell lying on its inner side. Only exceptionally, as in some species of Araceae, is cork in monocotyledons produced by a true initial cambium.

The tracheids in the secondary bundles of the banana rhizome elongate by sliding growth. Examination of a longitudinal section of a mature bundle shows that the numerous sieve tubes are all approximately of the same length; and the transverse sieve plates, with few exceptions, fall into definite groups very nearly in a plane. These groups evidently represent the positions of the end walls of the mother cell from which a segment of the bundle was derived. All the phloem cells between successive groups are evidently the products of the same mother cell. Only occasionally is an isolated sieve plate found between the groups in which the great majority of them lie, indicating that a transverse division has taken place in a sieve tube in the length of a single mother cell. The distance between the groups of sieve plates varies from 288 μ to 576 μ. Although the exact length of the tracheids in the secondary bundles has not been determined, they are very much longer than this. Hence it appears that the tracheids have elongated more than their sister cells, the sieve tubes, pushing into segments of the vascular bundle derived from other mother cells. SCOTT and BREBNER (9) conclude, from studies more especially directed to this point, that the tracheids of *Dracaena*, *Yucca*, and *Aristea* elongate by sliding growth.

In the obliquely downward course which they follow, the adventive roots may have to penetrate the solid parenchymatous tissue of the cortex, in the thicker portion of the rhizome, for a distance of 6–7 cm. before they reach the surface. The tips of roots at all stages in their progress through the cortex were examined, both in hand sections of fresh material and in solid blocks of tissue, cut so as to expose the growing point, and viewed under the binocular microscope,

and it can definitely be stated that a "digestive pocket," in the sense of a cavity in the tissue filled with fluid, is not present. The root cap touches on all sides the thin-walled parenchyma cells through which it pushes its way, and the cells directly before it are always somewhat crushed. They are, however, prepared for the penetration of the root by some digestive action, probably by enzymes secreted from the cells of the already massive root cap. The tissue immediately in front of the root tip, to the distance of about 1 mm., feels distinctly softer when touched with the point of a needle than elsewhere in the cortex, and collapses at the slightest pressure. The walls of cells not yet touched by the root cap are already distorted and show signs of breaking down. The amount of actually crushed tissue directly in front of the growing point is very slight, less than one would expect if no digestive action took place; and moreover the cells of the root cap themselves, in their rounded form, give no evidence of being subjected to pressure.

The amount of crushed tissue between the sides of the mature root and the surrounding cortical cells is variable. In some places there is practically no débris of collapsed walls, while elsewhere there may be considerable. On the whole, the absence of the walls of crushed cortical cells from large areas along the length of the root indicates that they have been dissolved by some enzymatic action which, however, is not complete. To summarize, the advance of the root through the tissues of the cortex is facilitated through the softening of the cells which oppose it, but this is accomplished by a digestive action which does not act with sufficient rapidity to dissolve their walls in advance of the root and form a free space or digestive pocket for its reception. The process of digestion, however, is continued until much of the débris of cell walls disappears. Normal living cells are not crushed by the root cap.

When it arrives within a few millimeters of the surface of the rhizome, the root tip broadens abruptly. It may in a distance of a few millimeters increase its diameter from 1.5 to 3 mm.

Courses and composition of vascular bundles

In order to follow the courses of the vascular bundles it was found necessary to differentiate them from the surrounding parenchyma by staining. To accomplish this, large suckers with broad leaves, and

bulbs in about the stage of that reproduced in figure 1, were selected and dug up in the early morning while the dew was still heavy on the leaves and the tracheids flooded with water. With a stout machete, the bulb of the sucker was first severed from the parent plant, and the roots were cut through in the soil at a distance of about 8 or 10 inches from the bulb. Then the bulb was dug up, leaving about the remaining length of the roots a ball of earth, which was later washed away under running water. After the bulb had been carefully cleansed, the sucker was tied upright against a support and the ends of a number of the best roots cut off again under water and at once inserted into test-tubes filled with the stain. Eosin and trypan blue, in 1 per cent aqueous solution, were both employed. Trypan blue, because it is strongly absorbed by the walls of the tracheids and diffuses hardly at all into the surrounding parenchyma, gives a much more precise differentiation of the bundle than does eosin. When neighboring roots of the same plant are placed one in eosin and the other in trypan blue, the eosin rises through the bundles much more rapidly, apparently because the other is so quickly removed from solution by the walls of the tracheids through which it passes that the water flowing upward soon becomes colorless.

After the roots had been about five hours in the stains, on a bright day a sufficient number of bundles was usually found to be colored. Attempts to trace the course of the leaf-trace bundles in longitudinally halved bulbs were invariably failures, since the bundles which enter the center of the bulb rarely complete their courses on one side. It was found necessary to begin at the top of an entire bulb and dissect downward, a laborious procedure. The course of a bundle was frequently so tortuous that a portion of it was inadvertently cut away while clearing a nearby length upon which it doubled back, and so its continuation was irretrievably lost. Only a single bundle could be followed in one bulb, since in tracing this bundle it was necessary to pare away much of the surrounding tissue.

The following types of bundles may be recognized. The general course of each may be seen by referring to the corresponding number in figure 3.

1. PRINCIPAL LEAF-TRACE BUNDLES.—These are the large vascular bundles, containing protoxylem elements, which are found in the

longitudinal septa of the leaf sheaths (10). Upon entering the bulb they follow an almost straight radial course through the cortex, penetrate the secondary zone (and the cambium in the case of the younger bundles), and proceed toward the center of the central cylinder. As they near the center their course becomes irregular and tortuous, with many baffling twistings and turnings. Some pass, in this indirect manner, across the bulb and finally lead to roots on the side opposite their point of entry; others, when they reach the center, turn to the right or left; and still others double back and connect with roots on the side from which they entered. In any case, as they again near the secondary zone they gradually turn downward and slowly converge with the secondary bundles. Finally, entering the secondary zone, they continue downward as secondary bundles, until they at length form connections with the roots.

The composition of these bundles changes greatly from one end to the other of their course through the bulb. As they enter it from the leaf sheath they are collateral. The xylem of each bundle contains several small protoxylem elements which are early disrupted and their lumina occluded by the ingrowth of the neighboring parenchyma cells, as described in an earlier paper (10), and by several metaxylem cells of medium size with spirally thickened, unlignified walls. The phloem with its occluded early sieve tubes is surrounded by a heavy sheath of fibers with very thick but unlignified walls, while a few thin-walled fibers occur around the xylem portion. Many sterigmata lie along the outside of the sheath of fibers. As the bundle passes inward through the cortex, the walls of the fibers become thinner, with the thickening restricted to the corners of the cells, and have larger lumina. By the time it enters the secondary zone the tracheids, which were previously arranged in a triangle, have spread out into a crescent, the thickening of the fibers is distinctly collenchymatous, and the sterigmata have disappeared (fig. 4). In the center of the rhizome the fibers accompanying each bundle are still further reduced in number and the thickening at the corners becomes very slight. The protoxylem is reduced to one or two elements usually not occluded. The metaxylem tracheids in this region are thickened by numerous, low-pitched spiral bands. Lignified elements are entirely absent.

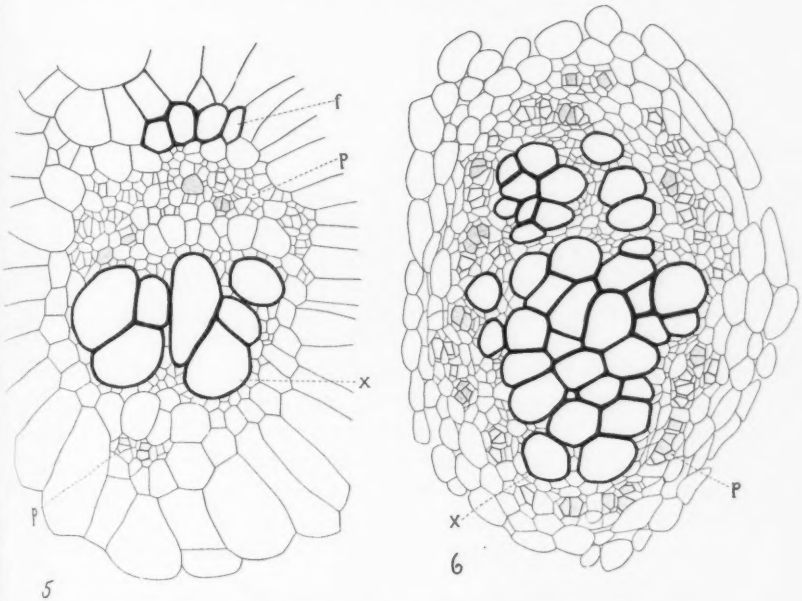
As the bundle passes downward and outward to meet the secondary zone, the protoxylem elements completely disappear and there is still further reduction in the number of fibers. As the bundle approaches the secondary zone, it acquires a few sieve tubes on its inner side, but still retains a few fibers (fig. 5). Upon entering the secondary zone, the phloem cells increase until they completely surround the xylem, while the fibers drop out of the bundle (fig. 6). The walls of the sieve tubes here as elsewhere are slightly thickened by broad, reticulated bars. Thus a collateral bundle equipped with protoxylem elements, fibers, and sterigmata gradually changes into an amphiphloic bundle devoid of any of these elements. The bundles differentiated from the cambium are merely the downward prolongations of the leaf-trace bundles, serving to form the connection between them and the adventive roots. They finally become part of the plexus about the base of a group of four roots.

Not all of the leaf-trace bundles which enter the central cylinder progress inward as far as its center. A large number (usually smaller bundles with fewer protoxylem elements than those just described or else none at all), after proceeding a greater or less distance into the central cylinder, bend rather abruptly to the right or left (the direction seems to be indifferent) and run for some distance in a tangential direction, girdling the bulb at the same time that they incline gradually downward. Finally they turn outward again and enter the secondary zone as amphiphloic bundles like those last mentioned. The tangential courses of these bundles cause them to be cut transversely in a longitudinal section of the bulb, such as figure 1, and the presence of the very numerous bundles encountered in cross-section in the intermediate zone of the central cylinder is at first sight a most confusing feature in the anatomy of the rhizome.

2. LONGITUDINAL SECONDARY BUNDLES.—These, as just explained, are the downward prolongations of the leaf-trace bundles. They are very large amphiphloic bundles of the type illustrated in figure 6. Together they form a zone at the outside of the central cylinder 3–6 mm. thick, in which the bundles occur three or four deep.

3. HORIZONTAL SECONDARY BUNDLES.—These, like the longitudinal secondary bundles, are very large and amphiphloic, often much larger and more complex than the longitudinal bundle chosen for

illustration (fig. 6). They form a plexus centering about the insertion of each group of four roots and anastomose with the longitudinal bundles, thus conducting water from the roots to the leaf traces. Since they form a system completely girdling the bulb, water enter-



FIGS. 5, 6.—Fig. 5, cross-section of leaf-trace bundle in lower portion of its course through bulb, on inner edge of secondary zone, showing transformation from collateral to amphiphloic bundle (note appearance of sieve tubes on inner side and great reduction in number of fibers). Fig. 6, cross-section of amphiphloic longitudinal secondary bundle. ×76.

ing through any one root can in case of need be distributed to the leaf-trace bundles ending on any side of the bulb. These bundles occupy a zone just outside the longitudinal bundles and more narrow than theirs, in which only one or two but occasionally three may be found on a radial line.

4. CENTRAL BUNDLES.—Connecting with the lower side of those leaf-trace bundles which penetrate to the center of the rhizome are certain slender bundles, devoid of protoxylem and prosenchyma ele-

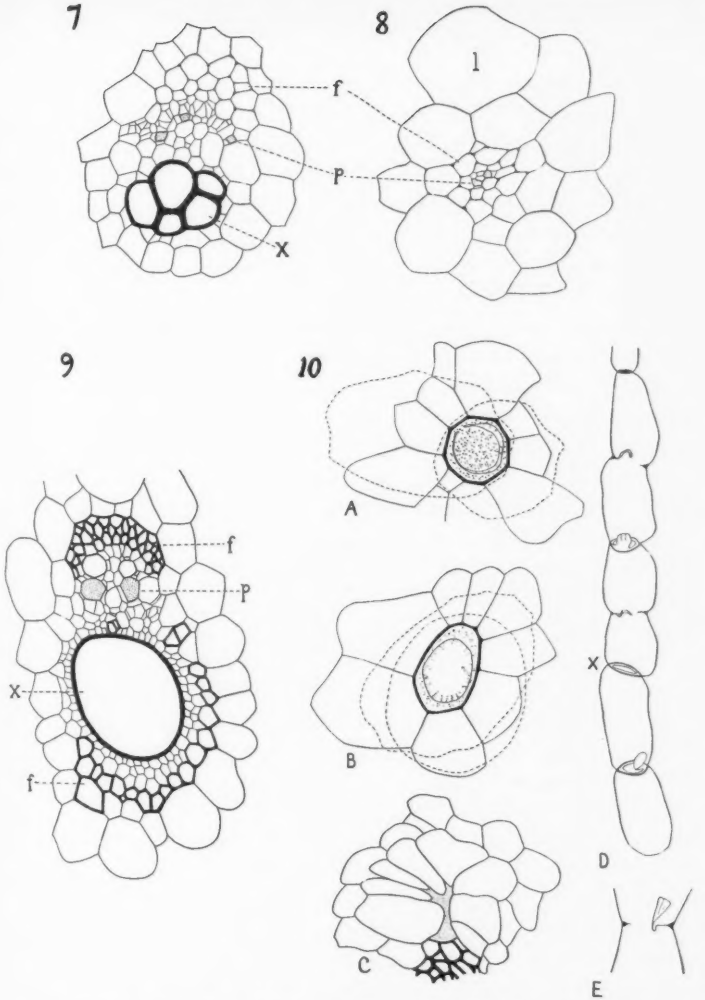
ments, and containing a few large tracheids, which run downward in an axial position but follow a rather flexuous course. Some at least continue until they enter the parent bulb.

5. CORTICAL LEAF-TRACE BUNDLES.—In the cortex there occur, in addition to the radially directed principal leaf-trace bundles, tortuous branching bundles whose general course is longitudinal. These vary in size from rather large bundles containing many tracheids, sieve tubes, and fibers (fig. 7 is a rather small but complete bundle) to very small bundles consisting of a few sieve tubes and thin-walled fibers, entirely devoid of tracheids (fig. 8). They finally enter the leaf sheaths, and hence form a second type of leaf-trace bundle. Their only communication with the roots is indirect and through the connections they form with the principal leaf-trace bundles as they pass radially through the cortex (fig. 3).

ENDODERMIS

As already mentioned, the short-lived cambium ceases its activity and its cells mature at a distance of between 3 and 4 cm. from the apex. At a later period an endodermis of an irregular type develops in the position previously occupied by it, between the horizontal secondary bundles and the cortex. The endodermal cells occur in an irregular series, interrupted by large gaps where the leaf-trace bundles penetrate it. They are nearly isodiametric, and do not differ greatly in shape from the cells of the ground parenchyma of the cortex and central cylinder, except that they are somewhat flattened tangentially. They are distinguished by the absence of starch grains (which are numerous in the surrounding parenchyma) and by the lignification of the middle lamella of the radial walls. This lignified strip differs from the usual Caspary band in the absence of the transverse corrugations so characteristic of the latter.

In the basal portion of the rhizome, far below the bases of the lowest living sheaths, the inner tangential and the radial walls of some of these cells become prominently thickened, lignified, and penetrated by numerous small, irregular pits. The thickening is always greatest on the inside wall (but here very variable even in cells close together) and tapers off on the radial walls in the manner typical of the endodermis of many monocotyledons. The occurrence of these



FIGS. 7–10.—Fig. 7, cortical leaf-trace bundle of medium size. ×84. Fig. 8, very small cortical leaf-trace bundle, destitute of xylem: *l*, latex vessel. ×120. Fig. 9, cross-section of vascular bundle from outer portion of central cylinder of aerial stem. ×76. Fig. 10, latex vessels: *A*, end wall between two component cells of latex vessel, showing central portion of wall remaining attached by narrow neck at one side; *B*, end wall between two cells, showing less usual case where central portion has broken away entirely (broken lines give outlines of central portions of two adjoining cells), ×166; *C*, latex vessel from older portion of rhizome, showing lumen almost occluded by ingrowth of surrounding parenchyma cells; *D*, portion of latex vessel: at *X* the end wall between the cells has completely disappeared while elsewhere it remains attached at one side, ×68; *E*, diagram showing end wall between two latex cells.

cells is extremely irregular: in places they form a continuous series; in others they are interrupted by endodermal cells with merely the middle lamella lignified; elsewhere in the same section they may lie singly between the thin-walled endodermal cells. Occasionally they are radially two or three deep, and in places they surround some of the outermost secondary bundles.

Epidermis, cork, and leaf scars

In the upper portion of a young rhizome the surface, except for the leaf scars, has a smooth, varnished appearance, and is of a deep cherry or mahogany color, variegated in spots with waxy yellow. The heavily cutinized epidermis persists until after the period of fruiting, although with increasing age numerous longitudinal fissures arise in it. The slow increase in diameter of the bulb stretches and flattens the epidermal cells until the lumen is almost or entirely obliterated. Their walls become irregularly lignified. The walls of the three or four outer layers of cells of the cortex become slightly thickened and impregnated with a brownish substance, apparently suberin, which renders them resistant to concentrated sulphuric acid.

The formation of cork is initiated beneath the cracks to which reference has just been made, and thence extends laterally until the older portions of the bulb are encircled by it. The cork originates just beneath the suberized cortical cells, in the fourth or fifth cortical layer from the exterior. The cork cells are not formed by a true initial meristem, but the activity of the first phellogen cells is limited, and after the cessation of its divisions its work is superseded by that of the next cortical cell lying toward the interior. Thus we have a "storied" cork or "Etagencork" such as Schoute (8) and Philipp (6) found to be characteristic of the monocotyledons. The total production of cork cells is at best slight, except at the leaf scars.

In each of the lowest lacunae of the older leaf sheaths is an accumulation of a hard, white substance, in part attached to the bottom and sides, and in part in small granules lying loose in the lacuna, the whole having a rather mealy appearance. This is composed of small, roundish cells freely proliferated from the bottom of the chamber. In the older living sheaths these cells are already suberized and resistant to the action of concentrated sulphuric acid. When the sheath

dies and decays, these masses of small cells continue to cover the portions of the scar which represent the bottoms of the lowest lacunae, and cork is not formed in these places. Elsewhere the outer cells of the scar are first suberized and later cork is formed beneath them. This is likewise a "storied" cork, but as many as 19 cells have been counted in a series derived from a single parent cell.

The lumina of the tracheids in the leaf-trace bundles beneath the scar are completely plugged by the ingrowth of the neighboring parenchyma cells in the form of tyloses. The tracheids in the most recent leaf scar are already occluded in this manner, and the entrance of fungi from the soil is thereby blocked.

Aerial stem

General features

As each shoot of the banana plant approaches maturity, the axis becomes more slender and produces longer internodes, pushing up through the center of the false-stem. Eventually it emerges from its upper extremity, when it is bent downward by the weight of the massive flower bud and so produces the pendent inflorescence and later the heavy bunch of fruit. Unlike the aerial stems of the majority of herbs, that of the banana is entirely too weak to hold itself erect (to say nothing of the fruit it bears) without the support of the numerous leaf sheaths which inclose it; for although of considerable thickness, it is almost entirely devoid of lignified fibers to impart the requisite strength. Removed from between the leaf sheaths it has a columnar appearance and is pure white outside and inside when fresh. On its lower half are inserted the largest leaves produced by the plant (**10,** table I). The internodes become increasingly long toward the top, and range from a few millimeters at the base, where there is gradual transition from the close-set nodes of the bulb, to about 90 cm. in length at the apex. In diameter the internodes range from about 5 to 8 cm. in the aerial stem proper. Each is thickest at its upper end, just below the insertion of a leaf.

In the aerial stem the central cylinder occupies practically the entire cross-section, while the narrow cortex is only 1–3 mm. thick. The ground tissue throughout is composed of thin-walled, practically isodiametric parenchyma cells, their walls penetrated by obscure,

fine, roundish or elliptical, simple pits, and rich in starch grains. In the central cylinder are many lacunae, which are most numerous in its central part, where they are narrow and long, attaining a length of 1 cm. or more.

No amphiphloic or secondary bundles are produced in the aerial stem, for these are found only in association with the adventive roots, and the last of them occur with the highest of these roots, at the point where the bulb narrows into the aerial stem. Neither does an endodermis arise in the aerial stem, but the boundary between the central cylinder and the cortex is marked by the presence of numerous crowded collateral bundles of the type represented in figure 9. Exterior to this ring of large bundles the cortical bundles are abruptly smaller. Passing outward in the narrow cortex, they show a gradual reduction in the already sparse xylem and phloem until strands composed of fibers alone lie near the epidermis.

Courses of vascular bundles

The courses of the vascular bundles in the aerial stem were determined, as in the case of the bulb, by placing the cut end of a stem, still surrounded by the leaf sheaths necessary to support it, in a 1 per cent solution of trypan blue. After the 24 hours or longer necessary to obtain good coloring of the bundles, the stem was removed from the stain and cut in halves lengthwise, and the bundles exposed by the use of a scalpel. Since the bundles follow a smooth and even course, this was an easier task than in the case of the bulb. Some of the bundles penetrate to the center of the stem. These may then continue across to the opposite side, or may again bend outward toward the side from which they entered. These two cases, it will be remembered, are also found in the bulb. Both the inward and outward courses are very gently inclined, and because of the great length of stem traversed by each bundle, it was not found possible to trace any one down to its connection with the roots, or until it reached the outer limit of the central cylinder the second time. Other leaf-trace bundles penetrate but a short distance into the central cylinder before again inclining outward toward the cortex. The courses of the bundles are not essentially different from those of the bulb, except for the great tortuosity of the latter, which appears to result from the abbreviation of the internodes.

As a vascular bundle enters the stem from a leaf, there is a considerable but temporary multiplication of the number of tracheids it contains, together with a marked reduction in their diameter. A typical case of a single bundle taken at various levels in the leaf sheath and stem will serve to illustrate the situation. At *A* (**10**, fig. 18), a point in the leaf sheath 8 mm. above its insertion, there was a single large functional tracheid and the overlapping ends of two narrow metaxylem tracheids. In addition there appeared the occluded lumen of a protoxylem element, and doubtless there were one or two smaller and earlier ones so completely obliterated that none of their widely separated bands of thickening appeared in the section. This bundle was here typical of the larger bundles throughout the leaf sheath. At *B* (the same bundle in the stem about 6 mm. below the insertion of the leaf), 14 distinct functional tracheids, large and small, were counted. The overlapping ends of two tracheids in the same longitudinal series are always counted as one, for were the bundle followed a short distance upward or downward, one or the other would fail to appear. The single large tracheid at *A* had about 2.7 times the cross-sectional area of the widest tracheid at *B*. At *C*, 5 cm. below *B*, the number of large tracheids was reduced to two, both much broader than the widest at *B*, and there were only four distinct functional small tracheids. At *D*, 38 cm. below the leaf insertion, there was a single very wide tracheid and a single narrow one (fig. 9), a condition again approaching that found in the leaf sheath.

Length of tracheids

A number of futile attempts to determine the length of the tracheids were made before a satisfactory procedure was found. Because of their great length, it is well nigh impossible to orient a longitudinal section with sufficient accuracy to include their entire extent. To isolate them by maceration is not practicable, because the extremely delicate membranes break down in the process and the spiral bands of thickening unravel. Cut stems were set into dilute suspensions of Higgins' American India ink, with the idea that as the water was drawn up into the tracheids by transpiration from the leaves above, the particles of carbon would be held back by the first end wall they encountered and so indicate its position; but these parti-

cles, which are invisible under the oil-immersion lens, proved so fine that they readily penetrated the walls and rose to a height of 60 cm. Finally the following method was successfully employed. A thin suspension of cornstarch in water was prepared, and the starch grains deeply colored by the addition of a solution of iodine in potassium iodide. A section of freshly cut stem was taken, cut in half longitudinally so that one end could be held between the lips while the other was immersed in the suspension, the stem being held as nearly horizontal as practicable. Upon sucking at the upper end the water was drawn into the broad tracheids, carrying along with it the starch grains, which accumulated at the upper end of those tracheids whose lower ends had been exposed by the cut. Upon uncovering the bundles by scraping away the surrounding parenchyma with a scalpel, and measuring the distance of the black mass of starch grains from the end, it was possible to determine the length of the tracheid. Of course, in only a small percentage of cases will the true length be obtained; for the tracheids will have been severed at all distances from their upper ends but only a small proportion will have just enough of the lower end cut away to permit entry of the starch grains, and these alone will reveal their full length. Accordingly only the maximum measurements of a series obtained by this method are significant.

A serious disadvantage of this procedure is that the starch grains burst through the end wall of a tracheid and enter the next above. If suction is continued longer, they accumulate in the upper extremity of the second tracheid until they finally burst through and enter the third, etc., and this in spite of the fact that the maximum pressure which could be exerted on these walls by suction is one atmosphere, while the actual pressure was undoubtedly very much less. No matter how light the suction, the starch grains broke through. The end walls of the tracheids are very oblique, and the spiral bands of thickening of the two tracheids separated by this wall are crossed, forming a grating with very fine meshes closed by the delicate cell membrane. The wall as a whole apparently was not broken down, but the starch grains appeared to have been forced through the meshes, tearing the membrane. This seemed to occur only after there was a considerable accumulation of grains, clogging the wall and building up a pressure.

Because of this difficulty, it was necessary to restrict measurements to the lowest accumulation of starch grains in each bundle, which indicated the position of the first end wall of a tracheid, where the mass of grains was always heaviest. Tracheids between 4 and 6 cm. long were found to be numerous in the aerial stem; more rarely they attain a length of 7 or even 8 cm.

This method of using starch grains, while difficult to apply in the case of the stem, where the bundles are crowded in a dense tissue and many contain a number of tracheids lying side by side, forms a satisfactory demonstration of the length of tracheids when applied to the bundles in the leaf sheath. Here the bundles in the longitudinal septa between the lacunae each contain a single large tracheid in cross-section, and the septa are so transparent that by cutting one out and holding it up to the light, it is easy to discern the individual bundles. If cornstarch is sucked up into the tracheids by the method described, and the suction is applied long and hard enough, the grains break through one end wall and then another until there is a black deposit in the upper extremity of each tracheid of a series of four or five, one above the other at rather regular intervals. The distance between successive deposits is readily measured and gives exactly the length of the tracheid. Using a low power magnifier (such as a watch-maker's lens), it is sometimes possible, upon inverting the piece of sheath and shaking gently, to watch the starch grains sink slowly through the entire length of the tracheid, thereby demonstrating that there is no wall between successive deposits. The wider tracheids in the sheath were found to vary in length from 3.8 to 8.0 cm. Those between 5 and 7 cm. long were numerous.

HABERLANDT (**2**) states in regard to tracheids: "Their average length rarely exceeds 1 mm., in the stems and petioles of *Musa* and *Canna* they may be more than 1 cm. in length (with a diam. of 0.08–0.1 mm.) while in the case of *Nelumbium speciosum* CASPARY has measured tracheids which are over 12 cm. long." The tracheids of *Musa* are actually many times as long as they have hitherto been supposed to be, and compare favorably in length with the longest tracheids known.

The largest tracheids in the aerial stem have lumina between 225 and 250 μ in diameter. The end walls are very oblique, and the over-

lap of two superposed tracheids in the same longitudinal series amounts to 5–10 mm. This broad overlap adds greatly to their water-conducting efficiency, for the greater the area of the membrane the less the resistance experienced by the water passing through it, at a given rate of flow in the tracheids.

The segments of the sieve tubes in the aerial stem are much shorter than the tracheids, and the longest encountered was only 1.2 mm. in length. Except at the top of the stem, where the light penetrates the leaf sheaths, the fibers surrounding the vascular bundles are thin-walled and unlignified, with the result that the stem, despite its considerable thickness, is weak and brittle.

Latex vessels

Since the latex vessels are a prominent feature of the anatomy of all parts of the axis, it seems most convenient to devote a separate section to them. Although varying greatly in degree of development in different regions, they are everywhere of the same type. They consist of cells joined end to end to form long vessels which occasionally branch (fig. 10 *D*). A single circular perforation, occupying almost the whole of the end wall between contiguous cells, gives free communication from one to another. The membrane which originally closed this orifice is not dissolved away, but rather the central portion is torn bodily from the edges. Usually it remains attached for a short distance at one side, and is folded back, more or less crumpled, into one or another of the two adjacent cells (fig. 10 *A*, *E*). Less frequently the wall is torn completely around and the central portion has entirely vanished, leaving only a narrow rim surrounding the wide perforation (fig. 10 *B*). Rarely an entire end wall remains between two cells in the longitudinal series. Both the circular flap, where this remains, and the persistent rim are perforated by numerous exceedingly fine pits. In a long series of cells making up a vessel, as a rule all the flaps are turned back the same way, suggesting that they have been forced in this direction by a common pressure.

In the bulb these latex vessels are found in both the cortex and the central cylinder, including both its primary and secondary regions. A cloudy fluid, which on exposure to the air becomes gummy, oozes slowly from them when the bulb is severed; and a steel knife used to

cut the bulb is soon blackened by the tannin they contain. A wide latex vessel lies on the exterior side of about half the longitudinal bundles of the secondary zone, but it may occur anywhere, and some bundles have two latex vessels associated with them. The chains of cells forming the vessels are here rather irregular (fig. 10 *D*) and the individual cells very large, ranging from 176 to 384 μ in length, by 64–144 μ in greatest diameter. In those latex vessels which follow the large leaf-trace bundles, the component cells are much elongated. In the cortex they attain 864 μ in length by 96 μ in diameter; in the secondary zone 1056×128 μ; in the primary region of the central cylinder 624×144 μ. In the older portion of the rhizome the lumina of the latex vessels are more or less occluded by the neighboring parenchyma cells, which stretch radially and push into them (fig. 10 *C*). Their content hardens to a firm, brownish substance which fills the remaining lumen.

In the aerial stem the latex vessels are of far less importance than in other regions of the axis. The exudation from them is scarcely noticeable when the stem is severed, and the knife employed neither turns black nor becomes fouled with a gummy substance, as in the case of the bulb. The tissue of the aerial stem contains so little tannin that it actually tastes slightly sweet. The latex vessels which occur throughout the cortex and central cylinder are rather narrow, being from 36 to 120 μ in diameter; but the component cells are long, attaining from 1.5 to 2.3 mm. in length. The proportions are accordingly significantly different from those of the segments of the latex vessels in the rhizome. It is of interest that the longest cells of the latex vessels of the aerial stem are about the same length as the longest segments of the sieve tubes.

It is in the rhachis of the inflorescence that the latex vessels reach their greatest prominence. If the rhachis is severed, the latex exudes in a steady trickle of cloudy drops, which on contact with the air soon become gummy. The rhachis of the male flowers is the only part of the plant where the latex vessels are as wide and prominent as the tracheids. Here their diameter reaches from 140 to 176 μ.

The fresh latex of the banana is a slightly cloudy or turbid liquid. The most prominent feature of its contents is the many sizable oil globules, which stain bright red in an alcoholic extract from the al-

kanna root. There are also many minute crystals which appear cubical but are so small that one cannot make certain of their shape, and extremely slender, rod-shaped crystals. The latex is immediately coagulated by a solution of ferric chloride, which imparts a black color because of the tannin it contains.

Summary

1. Close behind the apex of the bulb of the banana there develops a cambium-like meristem of limited duration, whose chief function is the origination of the adventive roots and the vascular bundles which link them with the leaf-trace bundles.

2. The number of tangential divisions which each cambium cell undergoes is rarely greater than seven, but the meristem is constantly regenerated from the adjacent undifferentiated cells of the inner cortex.

3. A single daughter cell of the cambium gives rise to an entire segment of a vascular bundle, as in the arborescent Liliiflorae.

4. The cambium gives rise first to longitudinal amphiphloic bundles, then to horizontal amphiphloic bundles and to the primordia of the adventive roots, which arise always in longitudinal rows of four. After initiating the horizontal bundles the meristem loses its activity, at about 3.5 cm. from the apex. The region of its activity extends for about 3 cm. in all.

5. The parenchymatous cells which stand in the way of the adventive roots pushing out through the cortex are softened by an enzyme secreted from the tip of the root, but a "digestive pocket" appears never to be present.

6. The strongest leaf-trace bundles penetrate to the center of the bulb. Thence they may pass outward toward the cortex in any direction, either by continuing across to the side opposite their point of entry, by doubling back to the side from which they entered, or by bending to right or left. As they approach the secondary zone on their outward course they incline downward and finally enter it. Their course through the central cylinder is everywhere extremely tortuous.

7. The longitudinal secondary bundles, formed from the cambium, are merely the downward prolongations of the leaf-trace bundles.

The same bundle which in the upper portion of its course is collateral becomes amphiphloic at its lower extremity.

8. The horizontal secondary bundles radiate from the bases of the roots and form a system girdling the bulb. Through their anastomoses with the longitudinal bundles they conduct water to the leaves.

9. The courses of the vascular bundles of the aerial stem do not differ essentially from those of the bulb, save that they are straight and regular.

10. The tracheids of both aerial stem and leaf sheath attain a maximum length of 8 cm. Those between 4 and 6 cm. long are numerous.

11. The latex vessels are formed of chains of large cells joined by a wide perforation occupying almost the entire end wall. The central region of the end wall is torn away from the narrow peripheral region, but usually remains attached at one side, forming a loose flap.

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LITERATURE CITED

1. ACQUARONE, PAUL, In Press.
2. HABERLANDT, G., Physiological plant anatomy. Trans. by MONTAGU DRUMMOND. pp. xv+777. London. 1914.
3. LINDINGER, L., Zur Anatomie und Biologie der Monokotylenwurzel. Beih. Bot. Centralbl. **19**:321–358. 1906.
4. MANGIN, L., Origine et insertion des racines adventives. Ann. Sci. Nat. Bot. Ser. 6. **14**:216–363. 1882.
5. PETERSEN, O. G., Remarques sur la croissance en épaisseur et sur les régions anatomiques de la tige monocotyledone. Bot. Tidsskr. **18**:125–126. 1892–1893.

6. PHILIPP, MARIA, Über die verkorkten Abschlussgewebe der Monokotylen. Bibl. Bot. **23** (**92**):1–27. 1923.
7. RÖSELER, P., Das Dickenwachstum und die Entwickelungsgeschichte der secundären Gefässbündel bei den baumartigen Lilien. Jahrb. Wiss. Bot. **20**:292–348. 1898.
8. SCHOUTE, J. C., Über Zellteilungsvorgänge im Cambium. Verh. Koninklijke Akad. Wetenschappen Amsterdam. Sect. II. **9**:1–60. 1902.
9. SCOTT, D. H., and BREBNER, G., On the secondary tissues of certain monocotyledons. Ann. Botany **7**:21–62. 1893.
10. SKUTCH, A. F., Anatomy of leaf of banana. BOT. GAZ. **84**:337–391. 1927.
11. ———, On the development and morphology of the leaf of the banana. Amer. Jour. Bot. **17**:252–271. 1930.
12. ———, Unrolling of leaves of *Musa sapientum* and some related plants and their reactions to environmental aridity. BOT. GAZ. **90**:337–365. 1930.
13. ———, Some reactions of the banana to pressure, gravity and light. Plant Physiol. **6**:73–102. 1931.
14. WHITE, P. R., Studies on the banana. Zeitschr. Zellforschung Mikrosk. Anat. **7**:673–733. 1928.

ANATOMY AND MICROCHEMISTRY OF THE COTTON SEED

R. G. Reeves and C. C. Valle

(with twenty-one figures)

Since the many uses of cotton-seed products have been recognized, the cotton seed, once regarded as worthless, has taken a prominent place among agricultural commodities. The process of extracting oil from the seeds presents various types of problems, and a great number of these problems remain unsolved.

We have very little knowledge of the histological changes involved in the extraction of oil, because the anatomy and localization of the chemical components of the embryo are not well known. Pentosans have been found in the cotton embryo by many investigators, and they sometimes make up a considerable part of the embryo. Occasionally the suggestion has been made that these pentosans occur in the cell walls. Xylan, one of the pentosans, is found in the cell walls of mature organs and tissues of many plants, especially after lignification of the walls. But the cell walls of embryonic structures have usually been found to be composed largely of cellulose. However, if the cell walls of the cotton embryo consist of cellulose, the localization of pentosans is still to be determined. Other structures in the cotton seed, particularly the palisade tissue of the seed coat, are likewise in need of investigation.

Among the publications dealing briefly with the anatomy and microchemistry of the cotton seed are those of Kobus (12), Harz (11), Bretfeld (5), Hanausek (9, 10), Winton (20), Balls (2), Stanford and Viehoever (18), Kondo (13), Wittmack (21), and Barritt (3); but none of these writers has given a thorough discussion of the subject. Further, investigators are in disagreement as to the structure of several of the tissues and as to the localization of certain of the chemical components. The present study was undertaken with the hope of acquiring a more accurate knowledge of the debatable questions pertaining to this subject.

Materials and methods

Most of the cotton seeds used in this study were from selected varieties of American upland cotton, *Gossypium hirsutum* L. The Mebane variety was studied most intensively, but Delfos, Half and Half, Rowden, and Acala were also used for comparison with Mebane. In addition, some seeds of Pima and Sea Island cotton were studied. Pima is variously regarded as *G. barbadense* L. and *G. peruvianum* Cav. It is commercially important in certain sections of southwestern United States. Sea Island is usually regarded as *G. barbadense*. Except where statements to the contrary are made, all of the data given will be applicable to mature seeds of any of these varieties of cotton. The drawings were made from the Mebane variety.

For the anatomical studies of immature seeds, the material was fixed in chromo-acetic-osmic solution, and stained in safranin or haematoxylin in the usual way. Sections were cut from 8 to 16 μ thick. For the anatomical study of the embryos of mature seeds, the same procedure was followed. The mature seed coats were found to be especially tough, however, and most satisfactory results were obtained by macerating with nitric acid and potassium chlorate, or by soaking the seeds in water for a few hours and then making freehand sections.

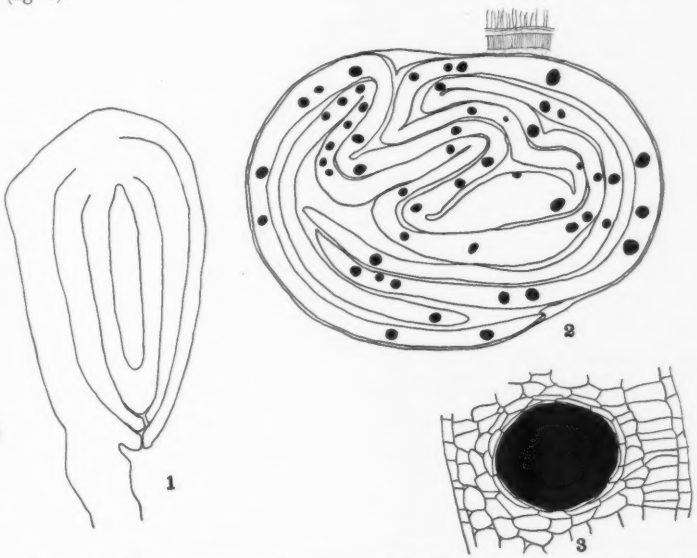
All of the microchemical studies were made with freehand sections. The methods used consisted chiefly of standard tests recommended by ZIMMERMAN (**22**), MOLISCH (**16**), and ECKERSON (**6**). In making the microchemical tests, the chief objective was to localize the cellulose, "lignin," pentosans, sugar, protein, and oil, all of which were already known to be present. Additional work was done to determine the presence and localization of starch in the seed. The microchemical tests were repeated a great number of times, and the results given here were regularly obtained.

Observations

YOUNG OVULE

The ovule is anatropous and has two integuments (fig. 1). At the time of fertilization its somatic cells are to a certain extent meristematic. Differentiation of tissues has not begun, except in the outer

integument where balloon-shaped protrusions can be seen. These are the first indications of the development of lint hairs. All of the cell walls of the ovule give a positive test for cellulose at this time. Soon thereafter differentiation of the tissues begins, and at the time of maturity of the seed some of these are highly specialized (fig. 2).



FIGS. 1–3.—Fig. 1, young ovule at time of fertilization, details omitted; ×18. Fig. 2, mature embryo with small sector of seed coat; ×11. Fig. 3, cross-section of cotyledon showing gland; ×112.

EMBRYO

The structure of the cotyledons of the mature seed is similar to that of the ordinary mesophytic, dicotyledonous leaf. The cotyledon is enveloped by an epidermis consisting of a single layer of cells in which undeveloped stomata are found.

The mesophyll consists of one or two layers of slender palisade cells and a spongy parenchyma several cells in thickness. Numerous glands, commonly called resin glands (fig. 3), may be found in the spongy parenchyma layer. The resin gland is surrounded by one or two layers of narrow flattened cells. The contents of the gland

stream out and show a lively Brownian movement when sectioned and placed in water. Treated with concentrated sulphuric acid they give a brilliant red color. When a section is treated with phloroglucin and hydrochloric acid, and heated, the contents of these glands give the red color characteristic of pentosans, and when treated with orcin and hydrochloric acid in the presence of heat they likewise give a positive test for pentosans. However, when a section is tested with phloroglucin and hydrochloric acid and allowed to remain at room temperature for about 20 minutes, the red color characteristic of the pentosan test occurs without heating. According to the procedure outlined by ZIMMERMAN (**22**) and ECKERSON (**6**) in testing for pentosans, this reaction should not occur at room temperature, but only after heating the preparation. If it occurs at room temperature the presence of lignin is suggested. The localization of lignin in glands would be a very singular condition, however, and the fact that the gland contents stream out in water is sufficient evidence that they do not consist of lignin. Heat hastens many chemical reactions, and there is no reason to doubt that the longer time required for this reaction to occur at room temperature compensates for heating.

The literature pertaining to the contents of these glands includes several suggestions that they contain gummy or oily substances mixed with resins. HARZ (**11**) states that the glands contain balsam. HANAUSEK (**10**) gives a short but interesting discussion of the "Schleim" in the cells surrounding the glands. There are numerous statements that these glands contain complex mixtures of substances.

According to MALOWAN (**15**), the quantity of pentosans in hulled cotton seeds is usually about 5 or 6 per cent, and other investigators have found approximately the same amount. The gland is the only part of the embryo that gives the slightest reaction of pentosans. As previously stated, the results obtained by making microchemical tests on the glands are not typical in every respect of pentosan reactions, but there is no reason to doubt that it is the contents of these glands which give the reaction widely recognized as a pentosan reaction by cotton-oil investigators. The test for pentosans used by MALOWAN and most other cotton-oil investigators is published by

the Association of Official Agricultural Chemists (1), and it is interesting to observe that this test also depends upon a phloroglucin reaction. The method used in the present study, as well as that adopted by the Association of Official Agricultural Chemists, depends upon the production of furfural from the pentosan and the subsequent reaction of phloroglucin with the furfural. Our results show that the only substance in the cotton embryo which can be regarded as a pentosan is found in the resin glands. STANFORD and VIEHOEVER (18) recorded no change after treating the resin glands with phloroglucin and hydrochloric acid, but they apparently did not heat their preparations, nor give them sufficient time to react.

Resin glands are also seen on the organs of the young cotton plant after the seed has sprouted. Those glands which are formed during the development of the seed remain after the seed sprouts, and perhaps additional ones develop. They are less conspicuous on normal seedlings than on etiolated plants, because the chlorophyll of normal plants tends to obscure them. Microchemical tests for pentosans were made on seedlings from one to 14 days of age which had been grown in darkness, and positive results were obtained on the glands of these plants. Pentosan tests were also made on normal green plants of corresponding ages and positive results were obtained with them.

STANFORD and VIEHOEVER (18) have shown some differences between the composition of glands that are exposed to light and those that are unexposed. They did not test the glands for pentosans, however, and therefore made no statements as to differences in pentosan content between exposed and unexposed glands. Further, all of the glands studied by the present writers were unexposed during development and none of them were exposed to light until the seeds sprouted. In this respect, the results of the present study are not comparable with those of STANFORD and VIEHOEVER.

The primary meristems, plerome, periblem, and dermatogen of the hypocotyl can be distinguished in the seed before it has sprouted, but mature tissues were not found. Glands similar to or identical with those of the cotyledons were also found in the periblem of the hypocotyl near its attachment to the cotyledons.

Oil was found in all of the tissues and organs of the embryo, but

was most abundant in the mesophyll of the cotyledons and the parenchymatous cells of the hypocotyl. It was found imbedded in the cytoplasm as minute droplets, and when sections were observed in water the droplets could be seen to coalesce, forming larger droplets. It was easily colored with Sudan III. Protein occurs as aleurone grains and as a component of the cytoplasm. It is very abundant and was identified with Millon's reagent, alkaline copper sulphate, and with nitric acid. It was found in all organs of the embryo. Of the several sugar tests used, only the Molisch reactions gave positive results. These positive results were probably caused by the trisaccharide raffinose, which was reported in the cotton seed by ONSLOW (17).

Tests for starch and oil were made on embryos of various ages, from the time the embryo was 3 mm. long until it was fully developed. An abundance of oil and small quantities of starch were found during this entire period. Small quantities of starch were found in a few mature seeds also. Most investigators who have analyzed cotton seed have reported an absence of starch. A review of the literature did not show a single instance of its being found in the embryo, but HANAUSEK (10) observed masses of small starch grains in the endosperm which is closely associated with the embryo. The amount of starch present in the mature seed is certainly very small, and its extreme scarcity is no doubt the reason for the common belief that it is entirely absent.

The starch grains found at maturity are very small and do not possess the lamellae characteristic of those of many other plants. They were found most frequently in the upper region of the hypocotyl. In many individual seeds it seems impossible to find starch grains, but occasionally a seed may be found that contains large masses of them.

Starch was found in all of the varieties and species of cotton studied. It was found in samples taken at random from large lots of cottonseed as it is ordinarily placed on the market. Starch grains were observed only after treating the embryos with potassium iodide, and when treated with this reagent they turned a distinct blue. The sections were then treated with chloral hydrate, which decomposed the other cell parts and caused the starch grains to swell.

It was found necessary to exercise unusual care in testing for starch in the cotton embryo, as certain other particles present often become dark after treatment with potassium ioiodide. They do not take on the blue color characteristic of starch, however, and are more uniform in size than the starch grains.

Endosperm

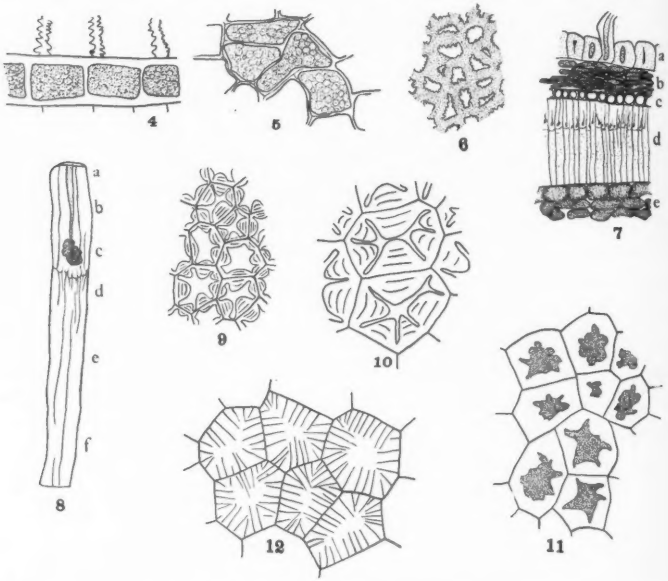
Soon after fertilization the embryo sac becomes lined with a layer of endosperm nuclei which lie free in the cytoplasm. Later these nuclei are separated from one another by cell walls which attain considerable thickness before the seed is mature. From the time the embryo is about 3 mm. in length the endosperm fills that portion of the embryo sac not occupied by the embryo. It is a soft, semi-transparent tissue and contains considerable starch. Most of this tissue is resorbed, however, and at maturity of the seed only a thin coat of endosperm remains around the embryo. This coat generally consists of a single layer of cells, but in furrows or spaces which are not occupied by the embryo there may be additional layers. The cells are rectangular in cross-section (fig. 4) and have rather thick walls which give the reaction for cellulose. At maturity they contain small quantities of starch and an abundance of protein and oil. In surface view they are irregular in shape (fig. 5). In general structure this tissue resembles the epidermis of the embryo to which it usually adheres.

Perisperm

The perisperm in turn adheres closely to the endosperm, and when examined with the unaided eye the two tissues seem to be a single structure. They often appear as a whitish sheath surrounding the embryo. When mature seeds are stored the embryo often shrinks until it becomes very loose in the hull. Under such conditions the perisperm and endosperm usually separate from the hull and remain attached to the embryo.

The perisperm consists of the remains of the nucellus. It is the epidermis of this organ and generally consists of a single layer of cells. At maturity its cells are highly differentiated. Its radial and transverse walls are very irregular, showing many toothlike projections which extend into the lumina (figs. 4, 6). The cells have no

tangential walls. This tissue has been designated by HANAUSEK (9) as "Franzenzellen," and by BRETFELD (5) as "dem Knospenkern hervorgegangenen Schichte." WINTON (20) refers to it as perisperm



FIGS. 4–12.—Fig. 4, section of endosperm and perisperm; ×600. Fig. 5, surface view of endosperm; ×600. Fig. 6, surface view of perisperm; ×310. Fig. 7, cross-section of seed coat: *a*, epidermis; *b*, outer pigment layer; *c*, colorless layer; *d*, palisade layer; *e*, inner pigment layer; ×85. Fig. 8, single palisade cell, longitudinal view; ×310. Fig. 9, cross-section of palisade cells near outer end, at *a* in fig. 8; ×310. Fig. 10, cross-section of palisade cells at *b* in fig. 8; ×600. Fig. 11, cross-section of palisade cells through masses of pigment at *c* in fig. 8; ×600. Fig. 12, cross-section of palisade cells at *e* in fig. 8; ×600.

and explains that it is the remaining epidermis of the nucellus. The cells of this tissue have no contents, and their walls are composed of lignin.

INNER PIGMENT LAYER

The innermost tissue of the seed coat is several cells thick. It is composed of thin-walled cells filled with a brown pigment (fig. 7 *e*) which first makes its appearance when the seed is almost mature. Long before the seed was mature an abundance of starch was found

in its outer portions. It first appeared to be most abundant in the outer integument, but in older seeds it was found in abundance in the inner integument also.

The starch causes the cells to stain darkly with safranin (figs. 14–17). In stained preparations the chalazal region of the inner integument is very striking. The cells are loosely arranged and are filled with starch grains. This region later develops into the thick, loose pigmented tissue designated by HANAUSEK (9) as the "Chalazagewebepolster."

The seed is white until it approaches maturity, but at this time its surface begins to change to a gray color. This change in color occurs at about the time the boll cracks, in its initial stages of opening. Seeds in this stage of development were examined microscopically, and the pigment that is abundant in the mature pigment layers was making its first appearance. The contents of the cells were still granular and had a dark tinge, but did not change in color upon treatment with iodine. This is the initial stage in the development of the pigment. After the change begins it continues rapidly until both of the developing pigment layers are affected. As the seed reaches complete maturity the seed coat loses much of its moisture and the cell contents become masses of hard brown material. The presence of starch in the undeveloped seeds of cotton has been previously reported by FLATTERS (7).

In mature seeds the cell walls of the inner pigment layer give a feeble positive reaction for lignin. During development they remain colorless and rather thin, and sometimes become loosened from their hard brown contents. This apparent dying and hardening of the protoplast always occurs in this tissue and in several other layers of the seed coat to be mentioned later. The contents of these cells gave negative results when tested for pentosans, oil, sugar, starch, and proteins.

Considerable variation is found in the thickness of the inner pigment layer in mature seeds. At the chalazal end of the seed and near the micropyle this layer is several times as thick as in other places.

PALISADE (MALPIGHIAN) LAYER

The palisade tissue is a single layer of greatly elongated cells which gives toughness to the seed coat. The cells are oriented so that

their long axis is perpendicular to the surface of the seed (fig. 7 *d*). The cell wall is so greatly thickened that no lumen is distinguishable except in the outer end of the cell. When a palisade cell is examined from lateral view, the wall is seen to possess various markings, many of them running lengthwise of the cell (fig. 8). At about one-third the length of the cell from the external end is also seen a series of short inward curved markings which extends across the cell. These markings which run crosswise of the cell indicate a region of mechanical weakness. When pressure is exerted on the tissue the cells always break in this region rather than elsewhere. Toward the outer end of the cell from this series of markings is found a small mass of brown pigment which is identical with that of the inner pigment layer just described. The portion of the lumen in which this mass of pigment is located is irregular in form. It has several sharp corners which extend radially toward the surface of the cell (fig. 11). The mass of brown pigment completely fills the lumen in this portion of the cell, and therefore often appears lobed. It sometimes tapers to a point toward the outer end of the cell. No nucleus or other cell parts can be recognized in the mass, although the mass is no doubt the remains of the protoplast. At maturity an empty part of the lumen extends from the mass of protoplasmic remains to the outer end of the cell.

BALLS (2) states that this brown mass of pigment consists of the remains of the nucleus of the cell. From the present study we are unable to determine that it consists of merely the remains of a nucleus. However, the mass probably contains the remains of the nucleus, along with some remains of the cytoplasm. Starch is common in the outer end of these cells before they are mature.

The structure of the individual palisade cell was found to be rather variable in its different regions, when examined in cross-section. The upper end of the cell has a very narrow but many pointed lumen, and the cell walls are greatly thickened except at the corners of the cells (fig. 9). The thickenings therefore appear as large lobes on the walls. The middle lamella and several secondary markings running parallel with the middle lamella can always be seen without staining when examined with the diaphragm of the microscope properly adjusted. Just above the mass of pigment the lumen is seen to be slightly smaller (fig. 10).

In the part of the cell where the mass of protoplasmic remains was found, no secondary lamellae in the cell walls were observed. The lumen is larger, and the lobed thickenings of the cell walls are less pronounced than at the point external to the mass of pigment (fig. 11). Along the middle portion of the cell the lumen is entirely closed, but many lines are seen running radially from the center of the cell to the surface (fig. 12). These lines probably represent the sharp points which were originally present in the lumina of the cell.

The "light line" of the palisade tissue of *Gossypium hirsutum* is a region at the upper end of the cells which contains a greater proportion of lignin than the adjoining region of the cell and shows a different refraction of light. It is not sharply distinguishable from the adjacent portion of the cell, and is not structurally differentiated.

When the palisade cells of *G. hirsutum* are treated with phloroglucin and hydrochloric acid, a positive test for lignin results in the extreme upper end and in the middle region of the cell (fig. 8 *a*, *e*). A slight reaction for lignin is usually obtained at *c* also, but the most intense reaction occurs in the middle zone at *e*. The zone marked *f* usually turns brown, but shows no definite indication of lignin. When the cells are cut transversely in region *e* and tested for lignin, the strongest reaction is found to occur along the axis of the cell or in the portion of the cell wall which was last laid down.

Studies of the seeds of Pima cotton showed that in this variety the region marked *a* contains no lignin, and *c* contains very little or none. But a very intense reaction for lignin occurs in *d* and *e*, and becomes gradually less intense in the inner end (*f*) of the cell. In Sea Island cotton a positive test for lignin was obtained only in regions *c* and *e*. Some differences are therefore apparent in the localization of the lignin in the palisade of these three groups of cotton. In this respect the difference between Sea Island and Pima is approximately as great as the difference between either of these varieties and Upland cotton, although Sea Island and Pima are often regarded as of the same species (*Gossypium barbadense*).

The localization of lignin in the palisade cells of several species of cotton has been recorded by KONDO (**13**), who worked with the variety Christopher's Improved of *G. hirsutum* and Mitafifi of *G. barbadense*. The results of the present study of Pima cotton agree in a

general way with KONDO's results on Mitafifi, and the present investigation of Upland cotton agrees with his study of that group. But our studies of Sea Island cotton (*G. barbadense*) do not confirm his work on that species.

In general, the portions of the cell which give no reaction or a very weak reaction for lignin give a strong reaction for cellulose. However, all parts of the cell wall gave positive tests for cellulose. When a section of the seed coat of any variety of *G. hirsutum* is treated with potassium ioiodide and sulphuric acid, the regions of the cell marked *b* and *d* are first to show a blue color. But after a few minutes the other portions of the cell also swell and turn blue. The outer ends of the cells swell most rapidly, and as a result this layer always coils inward before decomposition occurs. Likewise in Sea Island and Pima, regions *a* and *b* give a more distinct reaction for cellulose than any other part of the cell. These two regions in Sea Island and Pima are in no way differentiated from each other. In neither of the forms studied does the inner zone (*f*) give a strong reaction for cellulose, lignin, or pentosans. The strongest reaction observed in zone *f* occurred in Pima cotton.

The differences described here between the various groups of cotton in the localization of chemical components of the palisade layer were the only differences observed during this study. No doubt, however, there are many quantitative differences in the anatomy and chemical composition of the seeds of these species not recognizable by our methods of study.

Pentosans mixed with other substances probably occur in the palisade layers of all species studied. With the microchemical tests used in this work, however, it was difficult to distinguish between pentosans and lignin, since the same reagents are used in testing for both substances. Under ordinary conditions when the two substances do not occur together, it is possible to distinguish between them; that is, lignin gives a magenta red without heating and pentosans a cherry red only after heating. If pentosans are present, the two substances occur in the same general regions of the palisade layer. Pentosans are generally believed, according to ONSLOW (**17**), HAAS and HILL (**8**), and THATCHER (**19**), to occur in the seed coat of cotton. The pentosans of the seed coat are probably localized in the walls of these

palisade cells. The pentosan here present has been considered to be xylan, which usually forms a constituent of lignified cell walls.

Owing to the peculiar transverse markings observed when cross-sections of the seed are examined, the palisade tissue resembles a double layer of cells. By macerating the tissue, however, it is easily proved to be a single layer.

BARRITT (3) describes and figures "shoulders" on the cells of the palisade tissue. In regard to this tissue he states:

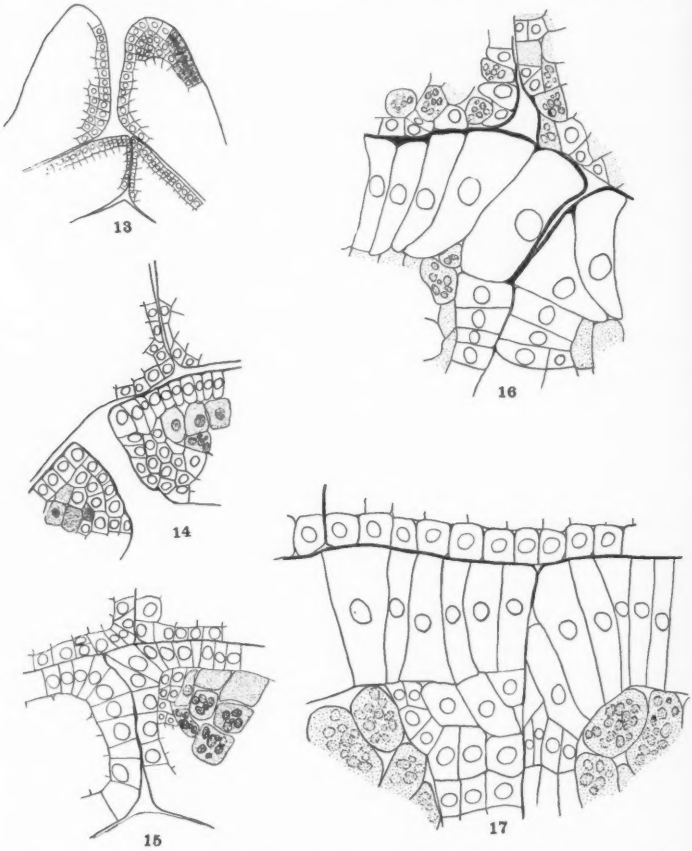
> Its cells fit very closely together from their inner ends up to about two-thirds of their length, at which point the cell walls bend inward, forming a shoulder and leaving intercellular spaces. This bottle-neck shape of the outer portion is only visible in thin sections in which a clear view is possible through the intercellular spaces.

But after carefully examining many sections cut very thin, as well as macerated tissues in which individual palisade cells were separated from one another, we failed to see anything that resembled shoulders. It is possible to account for BARRITT'S conclusions, however. The limits of the individual cells in this tissue are often difficult to distinguish on account of the many longitudinal markings which are sometimes more conspicuous than the middle lamellae. Striations running along the axis of the cell were mistaken by BARRITT for middle lamellae. On account of the lumen being open from the point where the mass of pigment lies to the end of the cell, BARRITT mistook pairs of this for an elongated intercellular space. He therefore mistook contiguous longitudinal halves of adjacent cells for entire cells.

BARRITT states further that the palisade layer is second to the inner layer of the outer integument. He regards the two integuments as showing "the typical leaf structures epidermis, palisade and mesophyll containing vascular tissue." The palisade layer already described in the present paper he regards as a part of the outer integument, and another palisade tissue he describes as occurring in the inner portion of the inner integument. He states, however, that this inner palisade tissue does not develop after the second week, and becomes crushed and disorganized by the developing embryo.

The present investigation has shown only one palisade layer in mature cotton seeds, and this is the outer palisade tissue. In incom-

pletely developed seeds, however, an inner palisade layer is also found, but this is resorbed before the seed is mature. Figures 13–17



FIGS. 13–17.—Sections through integuments at micropyle, showing successive stages in development of palisade layer and of starch. Fig. 13, ×140; figs. 14–17, ×310.

show that the outer palisade layer is the outer epidermis of the inner integument. The palisade layer of young seeds was examined very closely at the micropyle, where the inner epidermis of the outer integument is seen to turn outward with the edge of the outer integu-

ment, while the now feebly developed palisade layer turns inward, investing the mesophyll of the inner integument. The outer epidermis of the inner integument undergoes a slight differentiation into palisade cells even in the region where it lines the micropyle. The figures show further evidence of this in a slightly different way. The micropyle of the ovule consists of two orifices which often do not coincide (figs. 14–17), one passing through each integument. When the two do not coincide the palisade layer passes unbroken under the orifice of the outer integument and is interrupted only where the micropyle continues through the inner integument.

BARRITT is apparently the only investigator who has concluded that this tissue is a component of the outer integument. In plants of the Malvaceae other than *Gossypium*, LOHDE (14) in 1874 showed clearly that the palisade layer develops from the outer epidermis of the inner integument. The development in a plant of the Malvaceae of this tissue from the outer integument would be an anomalous phenomenon: there is no doubt that it is a part of the inner rather than the outer integument.

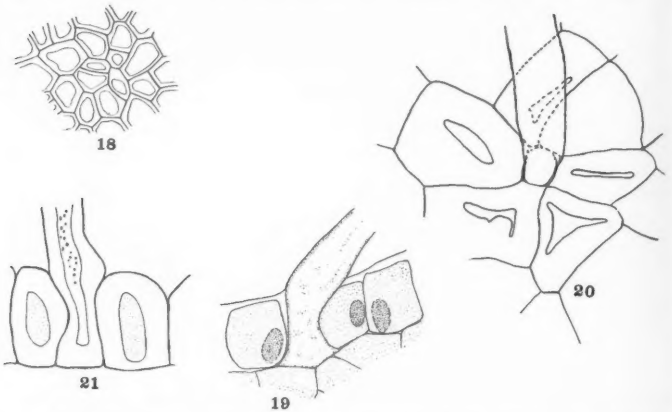
COLORLESS LAYER

The colorless layer (fig. 7 *c*), designated as the crystal layer by some investigators, is a tissue from one to two cells thick which incloses the palisade layer. Its cells have scanty or no contents and its cell walls are colorless. The walls are somewhat thick (fig. 18), and always give a positive test for lignin. The cells appear four-sided in sections and are approximately isodiametric.

OUTER PIGMENT LAYER

The outer pigment layer (fig. 7 *b*) is composed of brown pigmented cells which resemble those of the inner pigment layer, both in structure and in developmental history. This tissue is greatly thickened in the region of the raphe. BARRITT (3) advanced the hypothesis that the lint hairs obtain their nutrition from the liquids of the boll cavity rather than through the cells of the outer pigment layer. This boll cavity he regards as an intercellular space. From the results of the present investigation, it seems probable that the lint hairs obtain their nutrition through the cells of the outer pigment

layer. This tissue does not cease to function as living cells until shortly before the seed is mature. BARRITT's hypothesis cannot be verified by the results of the present study. The cell walls of the outer pigment layer are difficult to analyze microchemically. They appear to give a positive reaction for lignin, but the brown color of the tissue masks the color change. The contents of the cells gave negative results for all substances for which tests were made.



FIGS. 18–21.—Fig. 18, surface view of colorless layer; ×310. Fig. 19, section through young epidermis showing developing lint hair; ×415. Fig. 20, surface view of mature lint hair; ×310. Fig. 21, section through epidermis showing base of mature lint hair; ×310.

EPIDERMIS

The epidermis of the seed coat is composed of large, irregularly shaped cells with very thick walls composed chiefly of cellulose. The lumina are small and contain small quantities of brown protoplasmic remains (fig. 21). The cell walls themselves are also brown in color. The cells which give rise to the lint hairs appear much smaller in surface view than the surrounding cells, and are located in the center of a circle of these cells (fig. 20). The adjoining cells have a tendency to become wedge-shaped when observed in surface view, by reason of this peculiar arrangement. The mature lint hairs are constricted either just above or at the base. The origin of the lint hairs was not studied intensively; but in our observations, no

clear indication was seen of their arising from subepidermal cells, as BOWMAN (4) describes. The young hair often bulges or expands below the adjoining epidermal cells (fig. 19), so that if observed only in the mature condition it would appear to have arisen as a subepidermal cell.

The results of the microchemical tests are summarized in table I.

TABLE I

RESULTS OF MICROCHEMICAL TESTS OF MATURE COTTON SEEDS

SEED PART	CELLULOSE	LIGNIN	PENTOSANS	SUGARS	STARCH	OIL	PROTEIN
Embryo: resin glands	−	−	+	−	−	−	−
Embryo: cell walls	+	−	−	−	−	−	−
Embryo: cell contents	−	−	−	+	+	+	+
Endosperm: cell walls	+	−	−	−	−	−	−
Endosperm: cell contents	−	−	−	−	+	+	+
Perisperm: cell walls only	−	+	−	−	−	−	−
Inner pigment layer: cell walls	−	+	−	−	−	−	−
Inner pigment layer: cell contents	−	−	−	−	−	−	−
Palisade layer of seed coat: cell walls	+	+	+(?)	−	−	−	−
Palisade layer of seed coat: cell contents	−	−	−	−	−	−	−
Colorless layer: cell walls only	−	+	−	−	−	−	−
Outer pigment layer: cell walls	−	+	−	−	−	−	−
Outer pigment layer: cell contents	−	−	−	−	−	−	−
Epidermis: cell walls	+	−	−	−	−	−	−
Epidermis: cell contents	−	−	−	−	−	−	−

Summary

1. The cotton seed is composed of embryo, endosperm, perisperm, inner pigment layer, palisade (Malpighian) layer, colorless layer, outer pigment layer, and epidermis including lint hairs.

2. Traces of starch, in addition to oil and protein, occasionally occur in the cells of both young and mature embryos.

3. The substances commonly recognized as pentosans in the hulled seeds are located in the resin glands. These glands no doubt contain various other substances.

4. The cell walls of the embryo are composed chiefly of cellulose.

5. Starch, oil, and protein occur within the cells of the endosperm. The cell walls of this tissue are composed chiefly of cellulose.

6. Large quantities of starch are found in the two integuments of the developing ovule. It disappears, however, before maturity.

7. The pigmentation which occurs in the cells of the two pigment layers, and to a less extent in the palisade layer and epidermis, is associated with a hardening of the protoplast.

8. The palisade layer is a part of the inner rather than of the outer integument.

9. The cell walls of the epidermis are composed chiefly of cellulose. Those of the perisperm and colorless layer become lignified before maturity. In the two pigment layers the reaction was quite indistinct on account of the colors naturally present, but the walls appear to be composed of lignocellulose. The palisade layer of the seed coat contains cellulose and lignocellulose.

The writers wish to express their appreciation to Professor O. M. BALL for his encouragement and valuable suggestions during the course of this investigation.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 2d ed. Washington. 1925.
2. BALLS, W. L., The development and properties of raw cotton. London. 1915.
3. BARRITT, N. W., The structure of the seed coat in *Gossypium* and its relation to the growth and nutrition of the lint hairs. Ann. Botany **43**:483–489. 1929.
4. BOWMAN, F. H., The structure of the cotton fiber. London. 1908.
5. BRETFELD, F., Anatomie der Baumwolle und des Kapoksamens. Untersuchungen zum Zwecke der Construction von Verfälschungs, Identitäts- und Qualitätsdiagnosen. Jour. Landw. **35**:25–56. 1887.
6. ECKERSON, SOPHIA H., Microchemistry (manuscript).
7. FLATTERS, A., The cotton plant: its development and structure. London. 1906.
8. HAAS, P., and HILL, T. G., An introduction to the chemistry of plant products. New York. 1913.
9. HANAUSEK, T. F., Zur mikroskopischen Charakteristik der Baumwolleproducte. Zeitschr. Allgem. Österr. Apoth.-Ver. **26**:569–572; 591–595. 1888.

10. ———, Samen. Übersicht der Gewächse deren Samen technisch benutzt werden. In WIESNER, J., Die Rohstoffe des Pflanzenreiches. II. Leipzig. 1903.
11. HARZ, C. D., Landwirtschaftliche Samenkunde. II. Berlin. 1885.
12. KOBUS, J. D., Kraftfutter und seine Verfälschung. Landw. Jahrg. **13**:819–850. 1884.
13. KONDO, M., Über die in die Landwirtschaft Japans gebrauchten Samen, besonders über Malvaceensamen. Ber. Ohara Inst. landw. Forsch. [Kurdschiki] **2**:559–596. 1925.
14. LOHDE, G. L., Über die Entwicklungsgeschichte und den Bau einiger Samenschalen. Inaug. Dissert. Univ. Leipzig. Naumburg. 1874.
15. MALOWAN, J., Damaged cottonseed and seed analysis. Cotton Oil Press **5**:38–40. 1921.
16. MOLISCH, H., Mikrochemie der Pflanze. Dritte Auflage. Jena. 1923.
17. ONSLOW, M. W., Practical plant biochemistry. Cambridge. 1923.
18. STANFORD, E. E., and VIEHOEVER, A., Chemistry and histology of the glands of the cotton plant, with notes on the occurrence of similar glands in related plants. Jour. Agric. Res. **13**:419–435. 1918.
19. THATCHER, R. W., The chemistry of plant life. New York. 1925.
20. WINTON, A. L., The microscopy of vegetable foods. New York. 1906.
21. WITTMACK, L., Botanik und Kultur der Baumwolle. Berlin. 1928.
22. ZIMMERMAN, A., Botanical microtechnique (Transl. by HUMPHREY). New York. 1893.

MICROSPOROGENESIS AND CHROMOSOME BEHAVIOR IN NOTHOSCORDUM BIVALVE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 434

JOHN M. BEAL

(WITH PLATES II, III AND THREE FIGURES)

Introduction

Nothoscordum bivalve (L.) Britton occurs commonly throughout the southeastern portion of the United States, extending as far west as Texas and thence southward into Mexico. Being a member of the lily family, and showing evidences in its appearance of its affinities with the genus *Allium*, it was originally supposed that an examination of its chromosomes during meiosis might be of interest, especially since both parasynaptic and telosynaptic interpretations had been given for the chromosome behavior of species of *Allium*. Hence a few flower clusters were fixed and stained in the spring of 1925. Upon examination, these showed bivalent chromosomes of unusual form, but the press of other duties made it impossible to carry on the study further at that time. Subsequent fixations were made, however, and material was imbedded for future study.

Only three reports dealing with the chromosomes of *Nothoscordum* have previously been published, so far as the writer can determine. KOERPERICH (**16**) reported sixteen chromosomes as the somatic number in the root tips of *N. fragrans*, all of which show median or submedian constrictions. In *N. bivalve* ANDERSON (**2**) reported the haploid number as nine. He gives one figure of a dividing microspore nucleus which shows seven of the chromosomes with median or submedian constrictions and the other two with subterminal constrictions. In a preliminary paper (**3**) the writer confirmed ANDERSON'S count, and in general the types of constrictions reported. A brief summary and explanation are given for the unusual appearance which the bivalent chromosomes exhibit in meiosis.

Material and methods

All material used in this study was collected on the campus of Mississippi State College. Entire flower clusters, each containing

five or more flower buds in different stages of development, were collected just after they emerged from the bulbs. The two-valved spathe was carefully removed and the cluster of buds was placed in the fixing solution. On account of the small size of the flower buds it was not possible to remove the perianth segments from the individual flowers without causing injury to the anthers. The presence of the perianth possibly interfered to some extent with the penetration of certain of the fixatives, and this interference may account for the failure to secure good fixation in Flemming's solutions when used alone.

A variety of fixatives were used, the most satisfactory results being secured with a modification of NAVASHIN's solution, the formula for which was given me in a letter from Dr. KARL SAX. This solution consists of solution A (10 cc. glacial acetic acid, 1.5 grams chromic acid crystals, and 90 cc. distilled water) and solution B (40 cc. formalin and 60 cc. distilled water). Equal volumes of the two solutions were mixed just before using. Flemming's stronger solution, following a preliminary dip in Carnoy's fluid for a few seconds, also gave good results, especially for the late prophases and the subsequent stages in meiosis. Other fixations were made in Flemming's stronger and weaker solutions, in Benda's and Carnoy's solutions, and in chrom-osmo-acetic (1–2–100 chromacetic solution 9 cc., 2% osmic acid 1 cc.); but none of these fixations proved wholly satisfactory.

Sections were cut at thicknesses ranging from 8 to 18 μ, bleached in hydrogen peroxide when osmic acid had been used in the fixative, and stained, for the most part, in either iron-alum haematoxylin or Flemming's triple stain. Crystal violet-iodine was also used with very good success, but iron-alum haematoxylin on the whole gave the most satisfactory stains.

There is noticeable variation as between the anthers of a single flower as regards the phases of meiosis. One anther may show metaphases while another contains early prophases such as synizesis or the open spireme stages. Or one anther may show second division stages while the pollen mother cells of another are still undergoing the first division. There is also some difference as regards the two ends of the same locule, the apical end showing slightly more ad-

vanced stages than the basal end. These conditions make possible observations of several stages of meiosis in the same flower. And since the flowers of a cluster are in different developmental stages, it is possible to secure a number of meiotic stages in a single inflorescence.

Root tips were fixed in Flemming's medium solution, sectioned at a thickness of 10 μ, and stained with the triple stain or with iron-alum haematoxylin. This thickness was sufficient to secure entire groups of somatic chromosomes at the equatorial plate stage, and permitted a definite count of the diploid number for comparison with the number and morphology of those found in pollen mother cells.

Observations

Presynizetic stages

The anthers of *Nothoscordum* are very small at the time the sporogenous tissue can first be distinguished, averaging less than 1 mm. in length. Numerous cells throughout the anther show divisions which are apparently proceeding rapidly, accompanied by growth of the cells and by a corresponding increase in all dimensions of the anther. Soon, however, the premeiotic divisions in the sporogenous cells are completed and the definitive pollen mother cells enter upon their characteristic long period of growth.

In the resting condition the pollen mother cells are polyhedral and are easily distinguished from surrounding vegetative cells by their larger and more deeply staining nuclei and their denser cytoplasm. Just before the beginning of the prophases their nuclei average about 13 μ in diameter, and they show a variable number of nucleoli, two or three being common numbers. The chromatin is in the form of an irregular network composed of single strands (fig. 1). The strands are not uniform in thickness, but show much lumping of the chromatic material where they join or cross; these chromatic masses, however, show no indication of pairing nor do they suggest prochromosomes.

The mother cells and their nuclei now enlarge rapidly until the nuclei attain an average diameter of approximately 18 μ. During this period of enlargement rapid and marked changes occur in the chromatic reticulum. The strands become more thready and begin to

approximate in pairs (figs. 2, 3), while the lumps seem to flow out into the strands. Although the network shown in figure 2 is composed chiefly of single strands, there are several places where parallelism is now evident. In figure 3 the evidence of pairing is clearer, the strands having become more thready and the lumping of the chromatic material less evident. In fact, there is so much pairing of strands as to make it difficult to explain the condition as a chance approximation, nor does the parallel association strongly suggest the reassociation of the daughter halves of univalent chromosomes which had been split in the preceding telophases of the last somatic mitosis (DIGBY **9**, NOTHNAGEL **25**).

Shortly after the stage represented in figure 3, the reticulum begins to pull away from the nuclear membrane (fig. 4) and quickly passes into the condition of synizesis. During its rapid contraction, evidence of the pairing of strands is still clearer. There is some indication of twisting among the paired strands and the chromatic lumps have largely disappeared. The threads are now approximately uniform in thickness.

The synizetic knot lies toward one side of the nuclear cavity, usually closely applied to the nuclear membrane and commonly on the side nearest the mother cell wall. The nucleolus may be included in the knot, but many nuclei show it lying outside of and entirely free of the knot (fig. 5).

While these changes in the nuclear network are in progress a change is also taking place in the nucleoli. Whereas there may be as many as three or more nucleoli in the resting mother cell nucleus, never has more than a single large one been observed at synizesis or at later prophase stages. Apparently the smaller nucleoli either disintegrate or coalesce to form the single large one. Certainly a single large nucleolus is characteristic of all the stages from synizesis until its disappearance at late diakinesis. And whether one nucleolus or more is present, it never appears to be connected with the reticulum.

SYNIZESIS TO DIAKINESIS

Synizesis is apparently of short duration, a fact possibly correlated with the very rapid growth and development of the entire flower at this time. There is evidently a close and intimate association of the

synaptic mates during this close contraction. Thin sections show the tight knot to consist of compact but definite threads. Then, as the knot begins to loosen, threads are extended in the form of loops which show clearly their double nature (figs. 6, 7).

The double threads continue to disentangle themselves from the synizetic knot and extend out more and more until they reach the periphery of the nucleus to form the well known open spireme (fig. 8). The nucleolus appears to be free in the nuclear sap without connection with the spireme. In certain preparations the spireme threads, as shown in figure 8, appear to be single in the main, but careful observation shows that each is composed of two threads so closely twisted as practically to hide the double nature. Figure 9 is close in time of development to figure 8 and shows unquestionably the double threads, thus making clear the bivalent nature of the open spireme. In these two figures the chromatin appears to be evenly distributed along the double spireme. No free ends of threads, except those clearly due to cutting, have been seen in these stages, and these always show as paired ends.

Figure 10 represents the strepsinema stage. Here the twisted double threads are clearly evident, and are arranged in irregular loops. This sort of appearance would be a natural consequence of the arrangement of such an elongated, twisted, and loosely coiled thready structure.

The spireme threads now begin to shorten and thicken (fig. 11). As the condensation goes on there is evident a more or less noticeable massing of the double spireme across the central area of the nucleus to constitute what is known as the "second contraction" figure (fig. 12). However, this stage is not so evident as in the case of *Lilium* observed by ALLEN (1) and others. The loops extending from the second contraction mass are not significant in the formation of the bivalent chromosomes by a process of folding as a means of bringing alternately arranged maternal and paternal chromosomes into a side-by-side position. In agreement with ALLEN'S (1) observations on *Lilium canadense*, the segmentation of the spireme, already double, is mainly peripheral and not central. There are a number of free ends evident in or near the periphery of figure 12, but in every case they are double ends such as would result from the seg-

mentation of a double spireme. Figure 13 shows a slightly later stage, and it is difficult, if not impossible, to interpret this appearance as due to folding, for careful search for closed loops, which would support the telosynaptic interpretation, failed to disclose them in these stages and also in diakinesis.

A marked shortening and condensation of the spireme next occur without much increase in thickness of its double threads (figs. 14, 15), and transverse segmentation to form the bivalent chromosomes takes place. Both the figures just cited, as well as figure 13, show what at first examination were taken to be rows or chains of bivalent chromosomes being formed by segmentation of the double spireme, and which had not yet separated from one another. The upper group, in figure 15, shows a row of four such bodies. On further study, however, the conclusion has been reached that not all structures with this appearance represent simple bivalents. Certain of the bivalents are made up of pairs of homologous univalent chromosomes which show deep and prominent median or submedian constrictions. According to this interpretation the two constricted and paired threads in figure 14 represent a single bivalent chromosome, while the upper group of what appears to be four bivalent pairs in figure 15 is actually two pairs of bivalents showing the median or submedian constriction at the points marked *c*. Figure 16 shows a portion of another spireme in which the same condition is evident. There is further convincing evidence to support this interpretation. At early diakinesis (fig. 17) the bivalent pairs show the form of closed circles or "rings" of what might readily be mistaken for four univalent chromosomes joined end-to-end; also what appear to be bivalent pairs in the form of two crosses or of two parallel pairs joined end-to-end; these latter are much the more numerous. There are in each pollen mother cell nucleus seven such "rings" or double pairs and two ordinary bivalents.

ANDERSON (2) reports in a dividing microspore nucleus of *Nothoscordum* the presence of nine (n) chromosomes, of which seven show median or submedian constrictions and two show subterminal constrictions. My observations are in agreement with his so far as the median or submedian constrictions are concerned, but the subterminal constrictions have not been seen with certainty during meiosis.

There is evidence of subterminal constrictions in somatic chromosomes (fig. 30), but I have not been able to find them regularly on particular chromosomes. It is possible that the meiotic chromosomes in diakinesis and in the metaphases are so much shortened and condensed as to obscure the subterminal constrictions.

After the transverse segmentation of the double spireme is completed the chromosome pairs lie scattered irregularly throughout the nuclear cavity. They stain more intensely now than the spireme did in the preceding stages, while the nucleolus appears to be smaller and stains less intensely. The constricted chromosomes of each pair are still rather loosely joined, and the constrictions are very clearly evident. Figure 18 shows a group of bivalent chromosomes selected from a number of mother cell nuclei in diakinesis for the purpose of showing the various forms or configurations which are characteristic of this stage. Sometimes, as appears in figure 18*d*, the circle of four does not form a closed ring, one end of the pair being open. The constrictions show especially clearly at this time.

Diakinesis to equatorial plate

Toward the end of diakinesis the chromosome pairs begin to condense, the nucleolus disappears, and the nuclear membrane disintegrates. As the membrane disappears, fibrillae begin to extend into the nuclear cavity from the inner region of the surrounding cytoplasm. These fibers are at first more or less tortuous or wavy, and seem to arise from any portion of the circumference of the zone. Some of them seem to become attached to the chromosomes while others appear to connect the chromosomes.

The chromosomes become much more condensed, the two members of each bivalent pair coming into much closer contact. The four-parted appearance of the pairs of constricted chromosomes can still be recognized. The connecting fibers appear to shorten, while the radiating fibers stretch and straighten, the chromosomes thus being pulled into a rather compact, centrally grouped mass. Simultaneously with these changes the peripheral ends of the radiating fibers appear to move along the inner surface of the cytoplasmic region surrounding the chromosomes, and collect into three or more groups to form a multipolar spindle. These groups of fibers become

further shifted to form a bipolar, or, more accurately, a multipolar diarch spindle (fig. 20). During this development of the spindle the chromosome pairs are pulled into the positions characteristic of the equatorial plate stage.

Figure 19 shows a polar view of an equatorial plate from a preparation fixed in Carnoy's fluid. This figure shows clearly the seven apparently four-parted bivalents and the two bivalents of more usual form. This figure is typical of a great number seen clearly at this stage after treatment with each of the fixatives used.

Heterotypic metaphases to end of interkinesis

During the heterotypic metaphases the seven pairs of chromosomes with median or submedian constrictions show spindle-fiber attachments at or near the points of constriction; the other two pairs show terminal attachments (figs. 20, 21). None of the preparations show indications of a longitudinal split in any of the members of the pairs at these stages. However, as the chromosomes separate and move toward the poles a longitudinal split becomes evident, so that the single constricted chromosome of each disjoined pair again appears four-parted, the daughter halves lying near together in a more or less crosslike arrangement (figs. 22, 23), or more commonly as a double V. There is little lagging of chromosomes in any of the material, disjunction seeming to occur with general uniformity.

Upon reaching the poles the chromosomes are drawn tightly together with the free ends of the V's radiating toward the equatorial region. Nuclear membranes soon inclose the developing daughter nuclei, which grow in size, and the chromosomes become separated once more by clear spaces (fig. 24). This growth of the daughter nuclei is contemporaneous with the development of the cell plate. During the telophases the number of fibers between the two daughter nuclei appears to increase and their material seems to flow toward the equatorial region to form the cell plate. The development of a cell wall quickly follows. No indication has been seen of "extranuclear" nucleoles in the equatorial plate region at any time.

Each daughter nucleus lies very close to the cell wall; its outline, when the nuclear membrane first appears, is irregular, especially on the side toward the equatorial plane of the spindle. As the nucleus

grows it tends to become more rounded, but remains flattened during interkinesis on the side toward the equatorial region. The chromosomes do not lose their identity.

Homoeotypic division

The first evidence of a preparation for this division is seen in a further increase in size of the nucleus, accompanied by a loosening and spreading of the chromosomes. The nuclear membrane disappears and the spindle fibers appear in the clear area corresponding to the nuclear cavity. The two spindles lie most frequently parallel to each other, although they may vary slightly from this arrangement. The chromosomes in the equatorial plate (fig. 25) show the same variations in shape and the same general arrangement of daughter halves which were noted in the anaphases of the previous division. They become so closely crowded that a count is difficult at this stage.

The daughter chromosomes separate and begin their movement toward the poles (fig. 27). The median or submedian constrictions can still be seen clearly (fig. 26), although the chromosomes are now more irregular in form and more elongated than they were during the heterotypic anaphases. Upon reaching the poles the chromosomes shorten and become densely massed. The mass quickly becomes looser, however, and nuclear membranes develop about each of the four groups. Cell plates are now formed, as in the previous division (fig. 28), followed by the development of cell walls, and a quadrilateral tetrad of miscrospores results. The chromosomes soon become reticulate in appearance and the nuclei of the microspores develop a typical reticulum.

Chromosome number

For a time the apparently compound nature of a part of the bivalents caused trouble in making a definite count of the chromosomes, and not until root tips and dividing microspore nuclei were available could the count be made with certainty. Repeated counts at the heterotypic equatorial plate stage invariably show nine bivalents (fig. 19), and polar views of heterotypic anaphases confirm this count (fig. 23). Equatorial plates in the root tips show eighteen

chromosomes (fig. 29), and dividing microspore nuclei show the haploid number of nine (fig. 31), thus confirming the count. Furthermore, dividing microspore nuclei show that seven of the chromosomes have median or submedian constrictions and that the other two have very small subterminal constrictions or "satellites," facts which are in agreement with ANDERSON'S (2) report. Chromosomes in root tips show similar constrictions, but because of their crowding it has not been possible to locate the points of constriction in all eighteen. Selected individual chromosomes from a number of root-

FIGS. 29–31.—Fig. 29, equatorial plate stage from root tip, showing eighteen somatic chromosomes; median or submedian constrictions evident in some of the chromosomes. Fig. 30, eight chromosomes at diakinesis and the equatorial plate stages in mitosis showing the types of constrictions observed. Fig. 31, polar view of equatorial plate in a dividing microspore nucleus showing seven chromosomes with median or submedian constriction and two with subterminal constrictions or satellites. About ×1200.

tip nuclei are shown in figure 30. They illustrate the types of constrictions observed, but in none of the mitotic figures examined have as many as four chromosomes been found showing subterminal constrictions.

Discussion

CHROMOSOME CONSTRICTIONS AND RINGS

Chromosome constrictions are so widely known now in such a variety of forms that no attempt will be made here to list all the references. Suffice it to say that they are now generally recognized as normal occurrences. Among the many plants investigated, *Vicia faba* has probably been more closely studied than any other, and all recent investigators have observed the constricted chromosomes in this plant, although they were apparently overlooked in the earlier reports. FRASER and SNELL (10) and LUNDEGÅRDH (19) appear to

have observed constrictions in certain chromosomes, but seem to have attached no special significance to them. SHARP (**28**) showed constrictions (in his figure 32) but does not discuss them. SAKAMURA in 1915 (**26**), but more especially in 1920 (**27**), described in detail the constricted condition of the chromosomes both in somatic mitoses and in the reduction divisions. He also studied several other species of *Vicia*, finding constrictions in all of them except in *V. sativa*. He likewise reported constrictions in *Lathyrus vernus*, *Lens esculenta*, *Triticum monococcum*, *Zea mays*, and *Fritillaria camtschatensis*. NAVASHIN (**24**) described and figured the chromosomes for ten species of *Crepis*, in which all the chromosomes show constrictions. TAYLOR (**29**) reported constrictions in *Gasteria*, and in a series of later papers has shown them to occur in a number of other plants. ANDERSON (**2**) reported for *Allium stellatum* a haploid number of seven chromosomes, all of which show median or submedian constrictions, one of them showing also a subterminal constriction or satellite. Satellites, which are small terminal portions marked off from the rest of the chromosome by distinct constrictions, are also widely known and appear to be a constant feature of the chromosomes in a great number of organisms. But in none of the cases so far reported have the peculiar "rings" and double-appearing pairs observed in *Nothoscordum* been described.

The appearance of "rings" in *Nothoscordum* inevitably suggests the condition described in species of *Oenothera* by GATES (**11**), CLELAND (**7**), and other investigators, and in *Datura* by BLAKESLEE (**5**) and his colleagues. HÅKANSSON (**12**) reported a ring of four chromosomes joined end-to-end in *Pisum*, and a ring of three has been shown to appear at times in *Rumex acetosella* by KIHARA (**15**). In a recent paper HOAR (**14**) reports for *Hypericum punctatum* a condition very similar to that in *Oenothera*. In *Zea mays* also BURNHAM (**6**) and COOPER and BRINK (**8**) describe rings of four chromosomes in certain semisterile races. This occurrence is explained in accordance with the hypothesis of BELLING (**4**) as a consequence of segmental interchange between non-homologous chromosomes. COOPER and BRINK present good cytological evidence in confirmation of this view, which is supported also by MCCLINTOCK (**20**).

The interpretations which have been offered as explanations for

these conditions, while interesting and helpful in explaining certain genetical phenomena, can hardly apply directly to the "rings" in *Nothoscordum*, each of which consists clearly of only two chromosomes. But these interpretations may afford a clue for explaining the origin of the *Nothoscordum* "rings."

There are at least two ways of explaining the "rings" and apparent tetrads in *Nothoscordum bivalve*. First, as ANDERSON notes, in some *Allium* with a haploid number of eight a segmentation of one of the large median-constricted chromosomes might account for the haploid number of nine in *N. bivalve*. If such a break occurred independently at some division in microsporogenesis or in the microgametophyte leading to the development of the microgamete and in megasporogenesis or in the megagametophyte leading to the formation of the megagamete, fusion between two resulting gametes might have resulted in the development of a sporophyte with eighteen chromosomes instead of the sixteen of the parental stock. Or suppose the break to have occurred only once, perhaps early in the embryogeny of a plant. Such a plant would produce gametophytes of both sexes with the changed chromosomal condition, and self-pollination would result in the production of a zygote with two broken chromosomes, now four single ones. This hypothesis is given support by the fact that the two subterminally constricted chromosomes in the haploid set are each only slightly more than half the length of the longest chromosome with a median constriction.

Recently two species of *Allium*, *A. karataviense* and *A. triquetrum*, have been reported as having n=9 chromosomes. TELEZYNSKI (TISCHLER **30**) first gave the correct count for *A. karataviense*, which was confirmed by LEVAN (**18**), who also reported *A. triquetrum* as having nine as the basic number. LEVAN explains the condition in these two species as a result of the segmentation of one of the long median-constricted chromosomes of an eight-chromosomed species. This explanation was developed independently of ANDERSON'S (**2**) hypothesis of the origin of *Nothoscordum bivalve*. The constrictions shown in LEVAN'S figures are strikingly similar to those shown by both ANDERSON and myself for the chromosomes of *N. bivalve*, and add much support to this hypothesis.

It is likewise possible, of course, that *N. bivalve* may have been

derived from *N. fragrans*, rather than from an *Allium*, through the segmentation of a medianly constricted chromosome. But evidence as to which species of *Nothoscordum* is the more nearly primitive seems to be wanting, and until such evidence is available whatever may be said in this regard is purely speculative.

A second possibility is that the "rings" and apparent tetrads of chromosomes in *N. bivalve* have resulted from an end-to-end union in pairs of twenty-eight of the thirty-two chromosomes of a tetraploid species or race of *Allium*, of which several have been recently reported (MODILEWSKI **21, 22**; HIRATA and AKIHAMA **13**; LEVAN **17**; MORINAGA and FUKUSHIMA **23**). The condition in *N. fragrans* may be similarly explained by an end-to-end union in pairs of the entire thirty-two chromosomes of such a tetraploid form. KOERPERICH (**16**) reported only on the somatic chromosomes of *N. fragrans*, and whether the same appearance of "rings" and apparent tetrads of chromosomes occurs in this plant is not known.

In connection with this second hypothesis, it is necessary to assume that in some way these end-to-end attachments became permanent in order to account for the condition found in *Nothoscordum*. There is strong evidence in support of the view advanced to account for ring formation in *Oenothera*, *Datura*, and other plants to the effect that like ends of chromosomes tend to remain attached. It is possible on this basis to account for the end-to-end union of duplicate sets of parental chromosomes which would be found in a tetraploid *Allium* that came into existence in any one of the following ways: (1) through chromosome splitting without nuclear division in the first mitosis of the zygote; (2) through a fusion of the daughter nuclei following the first mitosis in the zygote; (3) through the union of diploid gametes following ameiosis; or (4) through hybridization between species in which the harmony between the sets of parental chromosomes is insufficient for the "pair action" necessary for normal reduction, resulting in a longitudinal splitting of all the chromosomes, the resulting halves then acting in pairs throughout the development of the resulting tetraploid sporophyte. As a result of any one of these four occurrences, it is conceivable that the duplicate pairs of chromosomes might be attracted to one another at the corresponding ends. If the attraction should be sufficiently strong,

twenty-eight of the thirty-two chromosomes might have remained permanently joined so as to show the median or submedian constrictions, while the four others, possibly as a result of gene mutations, failed to pair permanently, thus bringing about the condition observed in *N. bivalve*. Or if the attraction between all the duplicate pairs should be sufficiently strong, all the thirty-two might have remained permanently joined in pairs, resulting in the condition observed in *N. fragrans* by KOERPERICH.

Summary

1. The pollen mother cells of *Nothoscordum bivalve*, in the resting condition, show an irregular chromatin network which is made up of single strands with more or less lumping of the chromatic material where they join or cross.

2. During the early prophases the strands become more thready and begin to approximate in pairs. The pairing is clearly in accord with the scheme of parasynapsis rather than a result of the reassociation of the daughter halves of univalent chromosomes which were split in the preceding telophases.

3. As synizesis begins the pairing becomes more evident, with some indications of twisting between the threads. On emerging from synizesis the spireme is seen to be clearly double and much twisted. The doubleness persists in all the later prophase stages and the spireme appears to be continuous.

4. The spireme next shortens and condenses, becoming arranged into more or less well defined loops to form the "second contraction" figure.

5. Transverse segmentation of the bivalent spireme shortly begins to form the bivalent pairs of chromosomes. The loops are not significant in this process.

6. Other constrictions than those concerned with the segmentation of the spireme into bivalents become evident in the chromosomes at about this time, resulting in bivalent pairs of unusual form such as closed "rings" of what appear to be four univalent chromosomes joined end-to-end; also what seem to be two bivalent pairs attached end-to-end. There are in each pollen mother cell nucleus seven such "rings" or "double pairs" and two bivalents of the more

usual form. The apparent compound groups are really bivalents, each a pair of univalent homologous chromosomes which are deeply and prominently constricted in the median or submedian region.

7. Heterotypic division is regular and reductional. No evidence of a longitudinal split has been seen in any of the univalent elements during the metaphases or previously. The split becomes evident, however, at about mid-anaphase in all the disjoined chromosomes. There is little lagging of chromosomes.

8. A cell plate is developed by the phragmoplast in the equator of the mother cell during the late telophases, followed by the formation of a cell wall. Interkinesis is of relatively short duration.

9. Homoeotypic division is also regular. During the anaphases the constrictions are evident on seven of the nine chromosomes. Cell plates are formed in the equator of each daughter cell, followed by cell-wall formation, and a quadrilateral tetrad of microspores results.

10. Dividing microspore nuclei show nine chromosomes, seven of which have median or submedian constrictions, the other two showing small subterminal constrictions or satellites. Root-tip nuclei show eighteen somatic chromosomes with the same types of constrictions as are found in dividing pollen mother cell and microspore nuclei.

11. Two hypotheses are suggested for the possible origin of *Nothoscordum* from *Allium*.

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LITERATURE CITED

1. ALLEN, C. E., Nuclear divisions in the pollen mother-cells of *Lilium canadense*. Ann. Botany **19**:189–258. 1905.
2. ANDERSON, EDGAR, The chromosome complements of *Allium stellatum* and *Nothoscordum bivalve*. Ann. Mo. Bot. Gard. **18**:465–468. 1931.
3. BEAL, J. M., Chromosomes of *Nothoscordum bivalve*. BOT. GAZ. **93**:105–106. 1932.
4. BELLING, J., The attachment of chromosomes at the reduction divisions in flowering plants. Jour. Gen. **18**:177–205. 1927.
5. BLAKESLEE, A. F., Genetics of *Datura*. Zeitschr. Indukt. Abstamm. Vererb. Supplementband **1**:117–130. 1928.
6. BURNHAM, C. R., Genetical and cytological studies of semi-sterility and related phenomena in maize. Proc. Nat. Acad. Sci. **16**:269–277. 1930.

7. CLELAND, R. E., The reduction divisions in the pollen mother cells of *Oenothera franciscana*. Amer. Jour. Bot. **9**:391–413. 1922.
8. COOPER, D. C., and BRINK, R. A., Cytological evidence for segmental interchange between non-homologous chromosomes in maize. Proc. Nat. Acad. Sci. **17**:334–338. 1931.
9. DIGBY, L., On the archesporial and meiotic mitosis of *Osmunda*. Ann. Botany **33**:135–172. 1919.
10. FRASER, H. C. L., and SNELL, F., The vegetative divisions in *Vicia Faba*. Ann. Botany **25**:845–855. 1911.
11. GATES, R. R., A study of reduction in *Oenothera rubrinervis*. BOT. GAZ. **46**:1–34. 1908.
12. HÅKANSSON, A., Chromosomenringe in *Pisum* und ihre mutmassliche genetische Bedeutung. Hereditas **12**:1–10. 1929.
13. HIRATA, K., and AKIHAMA, K., Über die Chromosomenzahl bei einigen *Allium*-Arten. Bot. Mag. Tokyo **41**:597–600. 1927.
14. HOAR, C. S., Meiosis in *Hypericum punctatum*. BOT. GAZ. **91**:396–406. 1931.
15. KIHARA, H., Über das Verhalten der "end to end" gebunden Chromosomen vas *Rumex acetosella* und *Oenothera biennis* während der heterotypischen Kernteilung. Jahr. Wiss. Bot. **66**:429–460. 1927.
16. KOERPERICH, J., Étude comparative du noyau, des chromosomes et de leurs relations avec les cytoplasms (*Nothoscordum*, *Eucomis*, *Beschorneria*). La Cellule **39**:309–398. 1930.
17. LEVAN, A., Cytological studies in *Allium*. (A preliminary note.) Hereditas **15**:347–356. 1931.
18. ———, Cytological studies in *Allium*. II. Hereditas **16**:257–294. 1932.
19. LUNDEGÅRDH, H., Ueber Kernteilung in den Wurzelspitzen von *Allium cepa* und *Vicia Faba*. Svensk Bot. Tidskr. **4**:174–196. 1910.
20. MCCLINTOCK, B., A cytological demonstration of the location of an interchange between two non-homologous chromosomes in *Zea mays*. Proc. Nat. Acad. Sci. **16**:791–796. 1930.
21. MODILEWSKI, J., Zur Kenntnis der Polyembryonie von *Allium odorum*. Bull. Jard. Bot. Kieff **2**:9–17. 1925.
22. ———, Weitere Beiträge zur Embryologie und Cytologie von *Allium*-Arten. Bull. Jard. Bot. Kieff **7–8**:57–64. 1928.
23. MORINAGA, T., and FUKUSHIMA, E., Chromosome numbers of cultivated plants. III. Bot. Mag. Tokyo **45**:140–145. 1931.
24. NAVASHIN, M., Morphologische Kernstudien der *Crepis*-Arten in Bezug auf die Artbildung. Zeitschr. Zellforsch. Mikros. Anat. **2**:98–111. 1925.
25. NOTHNAGEL, MILDRED, Reduction divisions in the pollen mother cells of *Allium tricoccum*. BOT. GAZ. **61**:453–476. 1916.
26. SAKAMURA, T., Ueber die Einschnürung der Chromosomen bei *Vicia Faba* L. Bot. Mag. Tokyo **29**:287–300. 1915.

27. SAKAMURA, T., Experimentelle Studien über die Zell- und Kernteilung mit besonderer Rücksicht auf Form, Grösse und Zahl der Chromosomen. Jour. Coll. Sci. Tokyo Imp. Univ. **39**:1–221. 1920.
28. SHARP, L. W., Somatic chromosomes in *Vicia*. La Cellule **29**:297–331. 1913.
29. TAYLOR, W. R., Cytological studies on *Gasteria*. I. Chromosome shape and individuality. Amer. Jour. Bot. **11**:51–59. 1924.
30. TISCHLER, G., Pflanzliche Chromosomenzahlen. Tab. Biol. **7**:1931.

EXPLANATION OF PLATES II, III

All the figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Zeiss apochromatic objective, 2 mm. N.A. 1.40, with compensating ocular 15×. Magnification after reduction is approximately 1600.

PLATE II

FIG. 1.—Nucleus of young pollen mother cell showing the single strands with more or less lumping where they join or cross; two nucleoli show at this level.

FIGS. 2, 3.—Early prophases showing the pairing of strands (zygonema stages).

FIG. 4.—Beginning of synizetic contraction; paired strands are approximately uniform in thickness and some twisting is evident.

FIG. 5.—Mid-synizesis; single nucleolus present.

FIGS. 6, 7.—Spireme emerging from synizesis, with clear evidence of paired and twisted strands.

FIG. 8.—Open spireme; the association between the paired strands is apparently very close and intimate, but twisting and doubleness are evident in places.

FIG. 9.—Slightly later open spireme stage, with twisting and doubleness more evident than in figure 8; spireme threads cut in sectioning.

FIG. 10.—Strepsinema stage; twisting and doubleness clearly evident.

FIG. 11.—Tangential view of a nucleus at beginning of "second contraction."

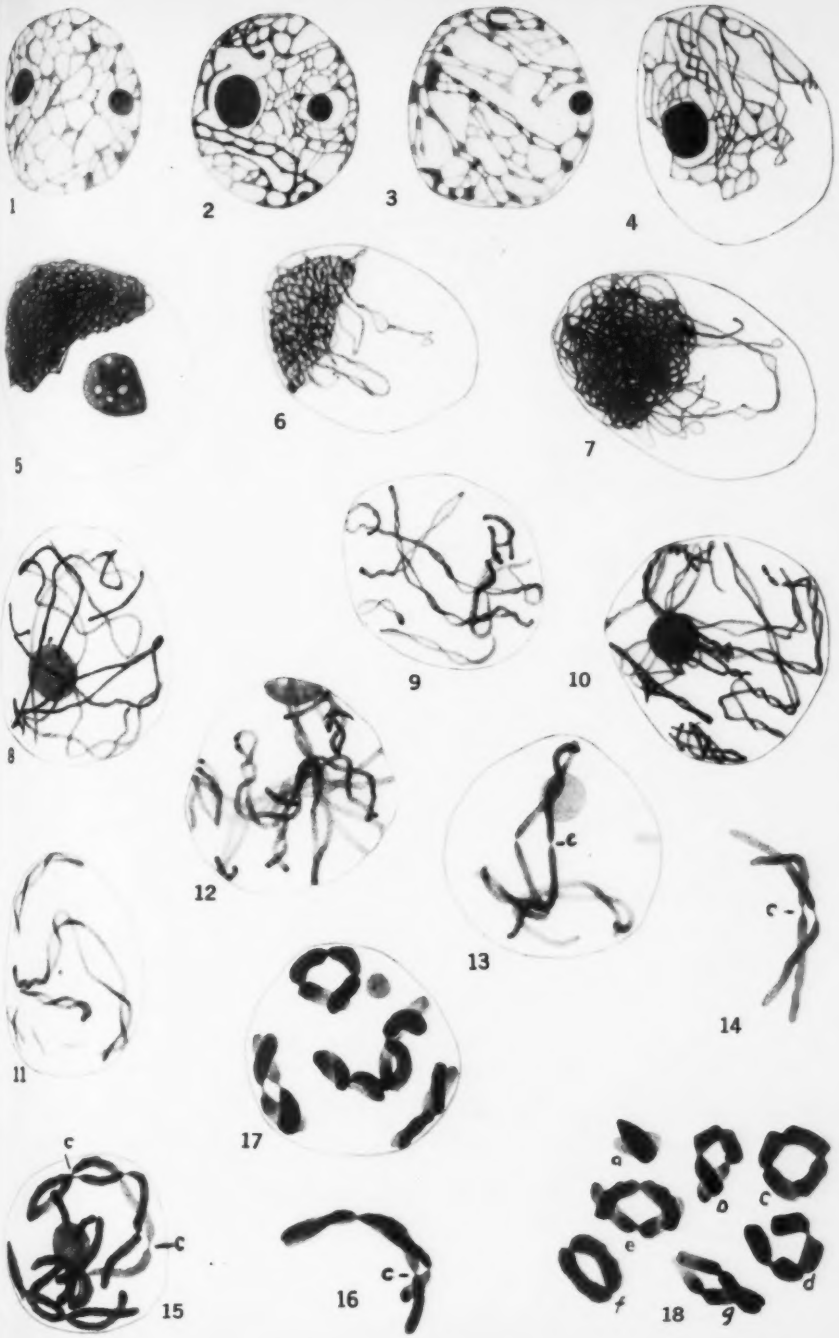
FIG. 12.—Second contraction figure showing looping and crossing of the bivalent spireme. Where the spireme is cut in sectioning the ends show as pairs of threads.

FIGS. 13, 14.—Selected segments of spiremes to show the constrictions which later become so evident in certain bivalent chromosomes; the spireme threads are somewhat more condensed than in figure 12.

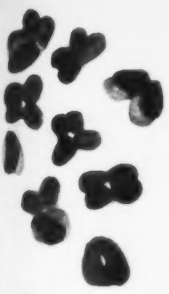
FIG. 15.—Spireme much more condensed and shortened; transverse segmentation into bivalent chromosomes is beginning, and median or submedian constrictions show clearly at points marked *c*.

FIG. 16.—Small portion of a spireme, stage of figure 15, with segmentation in upper portion and constrictions at *c*.

FIG. 17.—Portion of a nucleus at diakinesis showing one "ring" and four apparent tetrads. Each of these is a bivalent made up of pairs of homologous univalent chromosomes with prominent median or submedian constrictions.



BEAL on NOTHOSCORDUM



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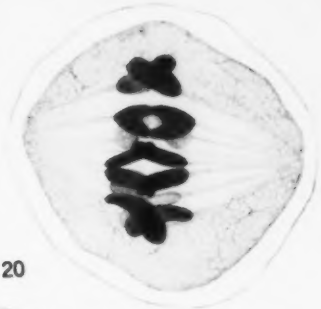

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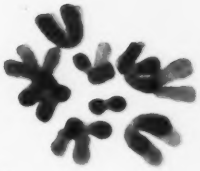

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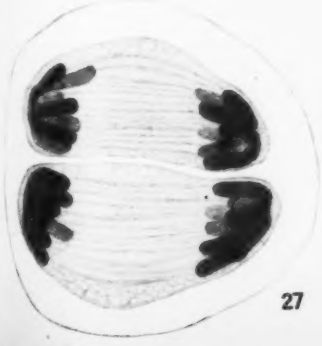

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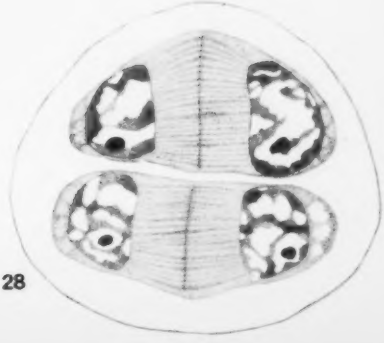

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FIG. 18.—Group of bivalent chromosomes taken from a number of nuclei at diakinesis to show the "rings" and double-appearing pairs. Two bivalents of the more usual form are shown at *a* and *f*, and a "ring" open at one end is shown at *d*.

PLATE III

FIG. 19.—Polar view of a heterotypic equatorial plate showing nine chromosome pairs. Seven of them are apparently four-parted while the other two show the more common form.

FIG. 20.—Early heterotypic metaphase showing the multipolar diarch spindle.

FIG. 21.—Group of bivalents selected from different cells at the heterotypic metaphases showing manner of disjunction and the spindle-fiber attachments.

FIG. 22.—Heterotypic anaphase; all the chromosomes show a longitudinal split.

FIG. 23.—Polar view of anaphase group in heterotypic division.

FIG. 24.—Interkinesis showing cell plate developing in the equatorial region of the pragmoplast.

FIG. 25.—Sister cells showing homoeotypic equatorial plates; division in the upper cell slightly more advanced than in the lower.

FIG. 26.—Four homoeotypic anaphase chromosomes showing median or submedian constrictions.

FIG. 27.—Homoeotypic telophase showing spindles lying parallel.

FIG. 28.—Late homoeotypic telophase; cell plates are developing and walls will quickly follow to form a quadrilateral tetrad of microspores.

THE ORIGIN AND BEHAVIOR OF CHIASMATA

I. DIPLOID AND TETRAPLOID TULIPA

C. D. Darlington and E. K. Janaki Ammal

(with ten figures)

After the chromosomes pair side by side in the pachytene stage of meiosis, they divide so that four chromatids are associated, and then fall apart in such a way that the four chromatids are differently paired in different parts, to give the characteristic diplotene loops. The points where they exchange partners are referred to as *chiasmata*, and probably their original position is, as we shall attempt to show, always interstitial, never terminal.

Between diplotene and metaphase, in some organisms, the frequency and distribution of the chiasmata seem to remain the same or nearly the same; in others the number of chiasmata is reduced and, as regards distribution, they are found to be in greater degree confined to the ends of the chromosomes as the prophase advances. In the limiting case the association of the chromosomes is purely terminal: they are joined end-to-end to form rings or rods.

Two questions then arise: How does this change take place? What relationship has the end-to-end association with the previous association by chiasmata? The answer that has been given (Darlington 5, and others) is that the change consists in a movement of the chiasmata away from the spindle attachment to the ends, of such a kind that they meet and fuse only at the ends and then never cancel one another. This movement is referred to as *terminalization*. It then follows, on developmental as well as on morphological evidence, that the end-to-end association is a chiasma; it is one in which the part of the chromosome distal to the exchange is reduced to zero; it is a terminal chiasma. This theory, which rested primarily on a comparison of the metaphase observations in *Hyacinthus* and *Tulipa* with those in *Tradescantia*, has several stringent requirements, which can be tested as follows:

1. Metaphase association of chromosomes, terminal or otherwise, will depend upon the frequency of interstitial chiasmata originally

TABLE I

TULIPA PERSICA: EARLY DIPLOTENE

NUCLEUS	BIVALENTS WITH			XTA		
	1 X*	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	2/ot	9/1t	1/1t	23	21	2
2	2/ot	8/2t	2/1t	24	21	3
3	2/ot	7/1t	3/2t	25	22	3
4	2/ot	7/2t	3/1t	25	22	3
5	1/ot	8/ot	3/3t	26	23	3
6	2/ot	7/1t	3/1t	25	23	2
7	1/ot	8/2t	3/1t	26	23	3
8	0/ot	9/2t	3/1t	27	24	3
9	1/ot	7/ot	4/2t	27	25	2
10	2/ot	7/1t	3/2t	25	22	3
Total	15	77	28	253	226	27
Mean Xta per bivalent				2.11	1.88	0.23
Chiasmata:						
Total	15	154	84			
Terminal	0	12	15			
Terminalization coefficient	0.000	0.078	0.179	0.106		

* In this and the following tables, X = chiasma, Xta = chiasmata, t = total number of terminal chiasmata. The terminalization coefficient is the proportion of the total chiasmata that are terminal at any stage.

TABLE II

TULIPA PERSICA: LATE DIPLOTENE

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	2/ot	8/2t	2/2t	24	20	4
2	2/ot	9/4t	1/1t	23	18	5
3	2/ot	8/3t	2/1t	24	20	4
4	2/ot	9/4t	1/1t	23	18	5
5	1/ot	9/4t	2/2t	25	19	6
6	1/ot	8/3t	3/2t	26	21	5
7	1/ot	9/3t	2/2t	25	20	5
8	1/ot	9/2t	2/2t	25	21	4
9	1/ot	9/3t	2/1t	25	21	4
10	1/ot	10/4t	1/1t	24	19	5
Total	14	88	18	244	197	47
Mean Xta per bivalent				2.03	1.64	0.39
Chiasmata:						
Total	14	176	54			
Terminal	0	32	15			
Terminalization coefficient	0.000	0.183	0.277	0.199		

TABLE III

TULIPA PERSICA: DIAKINESIS

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	1/ot	10/8t	1/2t	24	14	10
2	3/ot	7/7t	2/3t	23	13	10
3	2/ot	9/8t	1/1t	23	14	9
4	1/ot	9/4t	2/4t	25	17	8
5	3/ot	6/5t	3/4t	24	15	9
6	2/ot	7/3t	3/5t	25	17	8
7	2/ot	7/6t	3/3t	25	16	9
8	3/ot	7/6t	2/2t	23	15	8
9	2/ot	8/6t	2/3t	24	15	9
10	1/ot	8/5t	3/4t	26	17	9
Total	20	78	22	242	153	89
Mean Xta per bivalent				2.02	1.28	0.74
Chiasmata:						
Total	20	156	66			
Terminal	0	58	31			
Terminalization coefficient	0.000	0.371	0.469	0.368		

TABLE IV

TULIPA PERSICA: METAPHASE

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	1/ot	8/8t	3/2t	26	16	10
2	2/ot	9/9t	1/2t	23	12	11
3	2/ot	8/8t	2/3t	24	13	11
4	2/ot	9/10t	1/2t	23	11	12
5	2/ot	8/8t	2/2t	24	14	10
6	2/ot	9/10t	1/2t	23	11	12
7	2/ot	9/9t	1/2t	23	12	11
8	1/ot	10/9t	1/2t	24	13	11
9	3/ot	8/8t	1/2t	22	12	10
10	2/ot	9/9t	1/2t	23	12	11
Total	19	87	14	235	126	109
Mean Xta per bivalent				1.97	1.05	0.91
Chiasmata:						
Total	19	174	42			
Terminal	0	86	21			
Terminalization coefficient	0.000	0.471	0.500	0.465		

TABLE V

TULIPA AUSTRALIS: EARLY DIPLOTENE

NUCLEUS	BIVALENTS WITH				XTA		
	1 X	2 XTA	3 XTA	4 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	2/ot	7/2t	3/3t	0/ot	25	20	5
2	5/ot	4/1t	2/2t	1/1t	23	19	4
3	2/ot	7/3t	3/1t	0/ot	25	21	4
4	3/ot	5/3t	3/ot	1/1t	26	22	4
5	2/ot	6/3t	3/1t	1/1t	27	22	5
6	3/ot	4/3t	5/2t	0/ot	26	21	5
7	3/ot	5/2t	4/2t	0/ot	25	21	4
8	3/ot	7/1t	2/1t	0/ot	23	21	2
9	3/ot	5/1t	4/4t	0/ot	25	20	5
10	3/ot	7/3t	2/1t	0/ot	23	19	4
Total	29	57	31	3	248	206	42
Mean Xta per bivalent					2.07	1.72	0.35
Chiasmata:							
Total	29	114	93	12			
Terminal	0	22	17	3			
Terminalization coefficient	0.000	0.193	0.182	0.250	0.166		

TABLE VI

TULIPA AUSTRALIS: DIAKINESIS

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	2/ot	10/9t	None	22	13	9
2	2/ot	10/8t		22	14	8
3	3/ot	9/6t		21	15	6
4	3/ot	9/8t		21	13	8
5	4/ot	8/8t		20	11	9
6	4/ot	8/8t		20	12	8
7	4/ot	8/7t		20	13	7
8	4/ot	8/8t		20	12	8
9	4/ot	8/8t		20	12	8
10	4/ot	8/7t		20	13	7
Total	34	86	0	206	128	78
Mean Xta per bivalent				1.71	1.07	0.65
Chiasmata:						
Total	34	172	0			
Terminal	0	77	0			
Terminalization coefficient	0.000	0.437	0.000	0.378		

formed at diplotene. Failure of pairing will therefore be predictable in small fragment chromosomes and odd chromosomes with a reduced number of chiasmata (confirmed by DARLINGTON 3).

TABLE VII

TULIPA STELLATA: LATE DIPLOTENE

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	5/ot	16/5t	3/3t	46	38	8
2	7/ot	15/7t	2/ot	43	36	7
3	5/ot	15/8t	4/2t	47	37	10
4	4/ot	18/ot	2/2t	46	38	8
5	7/ot	14/5t	3/2t	44	37	7
Total	28	78	14	226	186	40
Mean Xta per bivalent				1.88	1.55	0.33
Chiasmata:						
Total	28	156	42			
Terminal	0	31	9			
Terminalization coefficient	0.000	0.198	0.214	0.177		

TABLE VIII

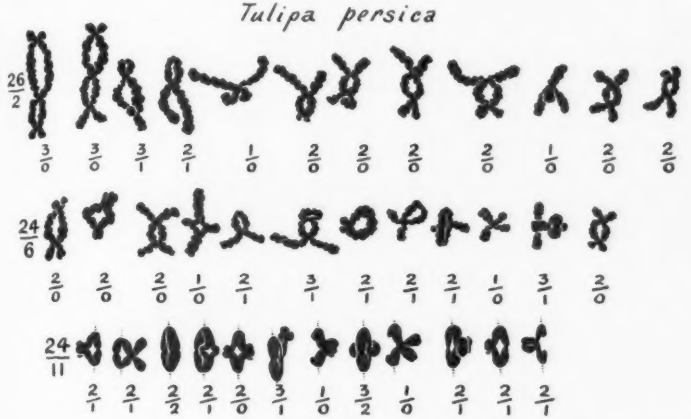
TULIPA STELLATA: METAPHASE

NUCLEUS	BIVALENTS WITH			XTA		
	1 X	2 XTA	3 XTA	TOTAL	INTERSTITIAL	TERMINAL
1	9/2t	13/13t	2/4t	41	22	19
2	5/1t	18/17t	1/2t	44	24	20
3	7/1t	16/16t	1/2t	42	23	19
4	7/1t	16/17t	1/2t	42	22	20
5	7/1t	14/13t	3/3t	44	27	17
Total	35	77	8	213	118	95
Mean Xta per bivalent				1.77	0.98	0.79
Chiasmata:						
Total	35	154	24			
Terminal	6	76	13			
Terminalization coefficient	0.171	0.493	0.542	0.446		

2. The appearance of terminal chiasmata will never be accompanied by an increase in total chiasmata and will usually be accompanied by some decrease owing to fusion. In other words, ter-

minal chiasmata will never appear except as derived from interstitial chiasmata and interstitial chiasmata will never disappear except through fusion with terminal chiasmata (confirmed by DARLINGTON 4, ERLANSON 7, PHILP and HUSKINS 13, GAIRDNER and DARLINGTON 8).

3. Multivalent configurations with terminal associations at metaphase in polyploids will show (a) multiple chiasmata where one



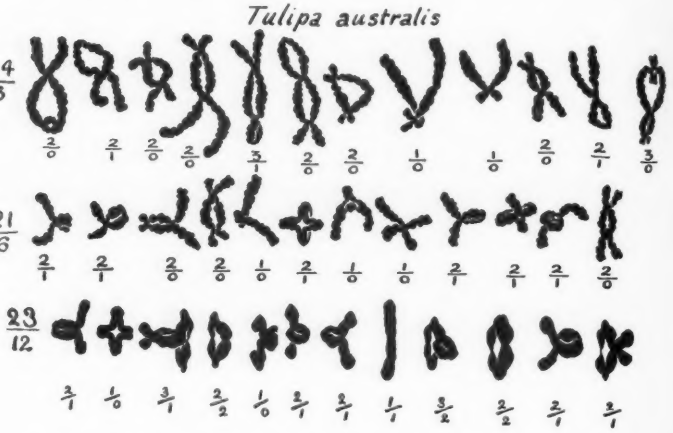
FIGS. 1–3 (fig. 1, above; fig. 2, center; fig. 3, below).—Bivalents of three nuclei of *Tulipa persica* at early diplotene, diakinesis, and metaphase; total number of chiasmata and the number terminal is given below each figure. ×1600.

chromosome has formed interstitial chiasmata with two others in the same arm; (b) random variation in the association or non-association of the ends of homologous arms according to the original randomness of their pachytene association conditioning chiasma formation (confirmed by DARLINGTON 4; cf. BELLING 1).

4. Where the movement is incomplete, chromosomes of similar length should show a smaller proportion of terminal chiasmata at metaphase when the number per bivalent is small than when it is large, because the average distance of the chiasmata from the ends is greater. (The proportion of the total chiasmata that are terminal at any stage we shall refer to as the *terminalization coefficient*.)

The exact determination of the changes in number and position

of the chiasmata during prophase is of immediate importance for two reasons. In the first place, accurate observations of chiasmata are so largely confined to metaphase (as in *Triticum*, *Pisum*, and *Hyacinthus*) that the original numbers of chiasmata can only be inferred from these, and this must depend upon an analogy with observations on material where all the stages can be studied. In the second place, an accurate study of the development of the metaphase bivalent will show whether there is any ground for supposing that



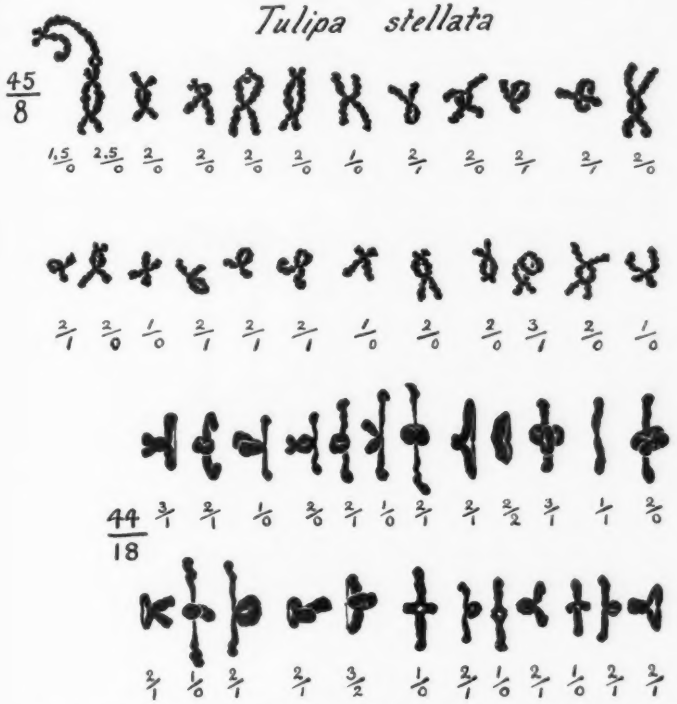
FIGS. 4–6 (fig. 4, above; fig. 5, center; fig. 6, below).—The 12 bivalents of three nuclei of *T. australis* at early diplotene, diakinesis, and metaphase; fig. 6 is from a different preparation and not strictly comparable with earlier stages. ×1600.

chiasmata break during terminalization. This hypothesis was included with several others by JANSSENS in his "chiasma type theory" of crossing over, and it is important to test these hypotheses separately since they are entirely independent.

The present study is undertaken in order to confirm the evidence of the second kind of test of the theory of terminalization, and to examine that of the fourth kind, which has not previously been considered. For this purpose smear preparations of the pollen mother cells of the diploid *Tulipa australis* Link (section Eriostemones), of its variety "*Tulipa persica*," and of the tetraploid species *T. stellata* Hooker (section Leiostemones) have been studied. These prepara-

tions were made by the late W. C. F. NEWTON (10) in continuation of his earlier study.

Changes in bivalent form between diplotene and metaphase are



FIGS. 7, 8 (fig. 7, above; fig. 8, below).—The 24 bivalents of each of two nuclei of *T. stellata* at late diplotene (with quadrivalent) and at metaphase. Quadrivalents and occasionally unpaired chromosomes also occur; failure of pairing is probably associated with chiasma frequency being lower than in the diploid (of which the larger number of free ends of chromosomes are an immediate indication). ×1600.

slight in *Tulipa* and have not hitherto been recorded (but cf. BELLING 2 and DARLINGTON 5). Three sources of error are present in this kind of observation:

1. The error of sampling: this has been reduced by taking a considerable series of observations and confining these to observations of whole nuclei in which all 12 bivalents are recordable.

2. The error of interpretation: this is less in *Tulipa* than in most plants because the material is exceptionally suitable for chromosome studies. The agreement between different series of observations as shown by the statistical arrangement indicates that the error is slight.

TABLE IX

SUMMARY OF OBSERVATIONS

TULIPA PERSICA (2n=24): 10 NUCLEI, 120 BIVALENTS AT EACH STAGE

	EARLY DIPLOTENE	LATE DIPLOTENE	DIAKINESIS	METAPHASE
1. Numbers of Xta:				
Total	253	244	242	235
Interstitial	226	197	153	126
Terminal	27	47	89	109
Terminalization coefficient	0.106	0.199	0.368	0.465
1a. Numbers of Xta per bivalent:				
Total	2.11	2.03	2.02	1.97
Interstitial	1.88	1.64	1.28	1.05
Terminal	0.23	0.39	0.74	0.91
2. Numbers of bivalents with:				
1 X	15	14	20	19
%	*12.5*	*11.7*	*16.3*	*15.8*
2 Xta	77	88	78	87
%	*64.1*	*73.3*	*65.0*	*72.5*
3 Xta	28	18	22	14
%	*23.3*	*15.0*	*18.3*	*11.7*
3. Terminalization coefficient with:				
1 X	0.000	0.000	0.000	0.000
2 Xta	0.078	0.183	0.371	0.471
3 Xta	0.179	0.277	0.469	0.500

3. When four stages of prophase are examined, the chiasmata seen in the later stages have arisen under conditions different from those seen in earlier stages. Since external or developmental conditions affect chiasma frequency, it follows that the different stages observed will never correspond exactly to the stages in the development of one nucleus; from this and from errors of sampling at a second analysis the discrepancies in table IX, 2 probably arise.

TABLE X

TULIPA AUSTRALIS (2n=24): 10 NUCLEI, 120 BIVALENTS AT EACH STAGE

	EARLY DIPLOTENE		DIAKINESIS	
1. Numbers of Xta:				
Total	248		206	
Interstitial	206		128	
Terminal	42		78	
Terminalization coefficient	0.166		0.378	
1a. Numbers of Xta per bivalent:				
Total	2.07		1.71	
Interstitial	1.72		1.07	
Terminal	0.35		0.65	
2. Numbers of bivalents with:				
1 X	29		34	
%		*24.2*		*28.3*
2 Xta	57		86	
%		*47.5*		*71.6*
3 Xta	31		0	
%		*25.8*		....
4 Xta	3		0	
%		*2.5.0*		....
3. Terminalization coefficient with:				
1 X	0.000		0.000	
2 Xta	0.193		0.437	
3 Xta	0.182		.....	
4 Xta	0.25		.....	

TABLE XI

TULIPA STELLATA (2n=48): 5 NUCLEI, 120 BIVALENTS AT EACH STAGE

	LATE DIPLOTENE		METAPHASE	
1. Numbers of Xta:				
Total	226		213	
Interstitial	186		118	
Terminal	40		95	
Terminalization coefficient	0.177		0.446	
1a. Numbers of Xta per bivalent:				
Total	1.88		1.77	
Interstitial	1.55		0.98	
Terminal	0.33		0.79	
2. Numbers of bivalents with:				
1 X	28		35	
%		*23.3*		*29.2*
2 Xta	78		77	
%		*65.0*		*64.2*
3 Xta	14		8	
%		*11.7*		*6.6*
3. Terminalization coefficient with:				
1 X	0.000		0.171	
2 Xta	0.198		0.493	
3 Xta	0.214		0.542	

Conclusions

1. Terminalization in *Tulipa* is incomplete. Between diplotene and metaphase some of the interstitial chiasmata become terminal.

2. The difference in distribution of chiasma frequencies at early diplotene in the two diploid forms suggests a difference in the degree of *interference* of the kind determined by HALDANE (**9**). In chiasma formation (fig. 9), *Tulipa persica* appears to have a higher inter-

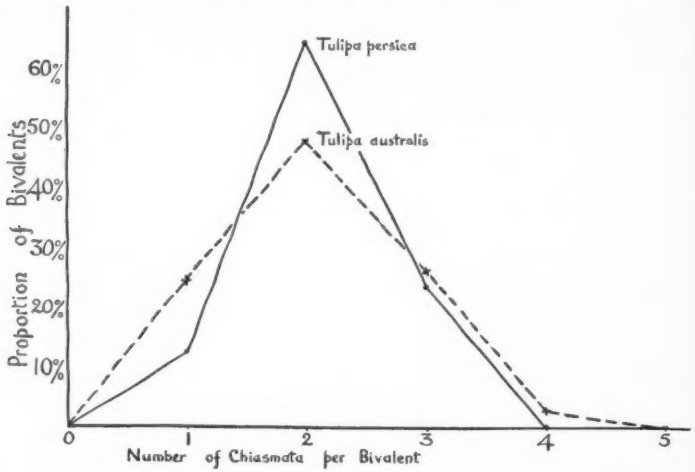


FIG. 9.—Distribution of chiasma frequencies at early diplotene in *T. persica* and *T. australis*, showing probably different degrees of chiasma interference.

ference than *T. australis*, although the mean number of chiasmata at diplotene is approximately the same. This cannot be associated with the lower terminalization coefficient, because at a later stage with a higher coefficient the discrepancy is sharper. The same kind of difference may be inferred in *Fritillaria* (DARLINGTON **3**).

3. The proportion of terminal chiasmata at every stage is lower, the lower the number of chiasmata in the bivalent; it remains at zero up to metaphase in those with only one chiasma in the diploid forms. This should mean that while three chiasmata are sometimes reduced to two, two are never reduced to one. It is found that in neither form is there any significant increase in the number of bivalents found

with one chiasma, while the number with two is increased at the expense of those with three. Expectation is therefore fulfilled.

An analysis of BELLING'S (2) observations shows the same behavior in *Lilium*. This can only be understood on the assumptions that terminal "junctions" are derived from chiasmata, a view which BELLING does not accept.

4. The increase in degree of terminalization is more rapid, the higher the number of chiasmata, especially in the early stages (fig. 10).

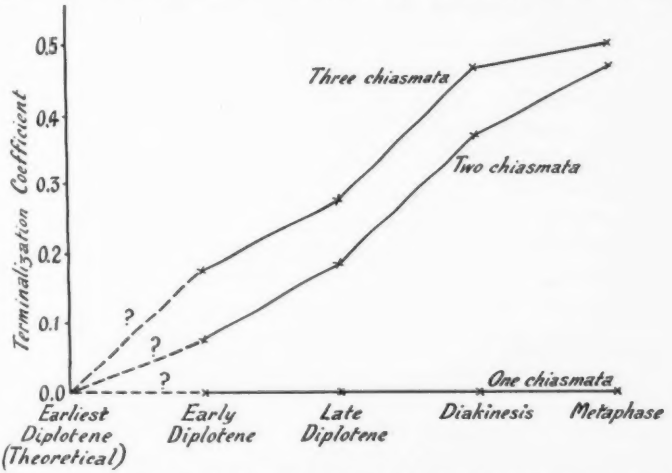


FIG. 10.—Change in terminalization coefficient in bivalents with different numbers of chiasmata in *T. persica*. Its original value is probably zero for all bivalents. The bivalents with different numbers of chiasmata at different stages do not exactly correspond owing to the reduction from three to two, but the effect of this is to reduce the apparent difference of behavior of the three types. (Cf. observations on *T. australis* and *T. stellata*.)

This is to be expected if movement of chiasmata is at the same rate in bivalents with different numbers of chiasmata, for the higher the number of chiasmata the nearer to the end the distal one is likely to be, if they are distributed at random. But the difference in terminalization coefficient between those with one and those with higher numbers is so sharp that randomness alone will not account for it. It must be assumed, either (1) that the rate of movement of single chiasmata is less than where there are several; or (2) that single

chiasmata are always formed near the middle of the chromosome (probably near the spindle attachment, which is submedian in most chromosomes); or (3) that both these conditions affect the result. This question will be considered in greater detail elsewhere on the basis of evidence in other organisms.

5. Metaphase in *Tulipa australis* has not been found in the same preparation as prophase. Figure 6 shows it not to be comparable, having a higher chiasma frequency than is expected and terminalization of single chiasmata. Environmental, as well as genetical, conditions have earlier been shown to affect chiasma frequency and are doubtless operative in this case. Comparative records of this stage have not therefore been made.

6. The different kinds of analysis to which the observations have been submitted fail to show any significant evidence of the breakage of chiasmata. Such evidence would consist in a reduction in the number of chiasmata otherwise than by fusion of chiasmata at the ends. The most striking evidence of this is found in *Tulipa australis* (table X), where it is seen that while the bivalents with two chiasmata increased in number owing to the fusion of chiasmata in those earlier having had three and four, those with one did not increase (cf. paragraph 3). This remarkable difference is necessary on the terminalization hypothesis because none of those with one chiasma had this chiasma terminal: no reduction from two to one could be supposed to have taken place. On the breakage hypothesis this property could not be predicted. Other evidence against the breakage hypothesis has been described in considering the occurrence of interlocking[1] (GAIRDNER and DARLINGTON 8).

[1] This hypothesis has recently been extracted from the chiasmatype theory and revived as a single explanation of crossing over by SAX (14,15). It cannot be examined as a working hypothesis, however, without a statement of: (1) what proportion of interstitial chiasmata may be supposed to break (although he implies that all may break); (2) what proportion of terminal associations may be supposed to arise *de novo* (an occurrence which we deny) and what proportion may be derived by terminalization of chiasmata; and (3) what conditions may be supposed to influence these occurrences. Apparently a single hypothesis which will account for the development found with incomplete terminalization in *Tulipa* and that found with complete terminalization in *Campanula* in any other way than that we have described will need to be highly elaborate. It cannot be at the same time supposed that it will account for genetic crossing over, since organisms with similar conditions of crossing over may have complete terminalization (as in *Primula*) or almost complete absence of it (as in *Lathyrus*).

7. General considerations favor the view that chiasmata arise at the earliest stage of diplotene always interstitially and never terminally. Thus (1) since there is an increasing number of terminal chiasmata at the later stages and only a small number at the earliest recordable stage, it is easier to suppose that the early terminal ones arose by early terminalization, rather than that terminal chiasmata arise both primarily and secondarily. (2) It is necessary to assume that the high proportion of terminal chiasmata in the fragments of *Fritillaria* is not due entirely to different conditions of chiasma formation but to different conditions of terminalization in short chromosomes, since the frequency corresponds with that of the interstitial chiasmata in the large chromosomes. (3) It is difficult to understand why a terminal exchange of partner (chiasma) should occur in some chromosomes and not in others, except through differences in the terminalization of interstitial chiasmata. (4) On the simplified chiasmatype hypothesis (DARLINGTON **4**), chiasmata cannot be supposed to arise other than interstitially. The changes in terminalization coefficient shown in figure 7 support this conclusion, for the coefficient of each of the three types is most easily derived from a zero value at a slightly earlier stage than the earliest recorded.

8. The observations on *Tulipa* indicate the conditions of terminalization and the amount of change in other organisms with similar terminalization coefficients at metaphase (for example, *Pisum*). A comparison of the available observations on terminalization shows that with the same chiasma frequency a higher terminalization coefficient is associated with a greater reduction of chiasmata during prophase. This means that the short-chromosome race of *Matthiola* must have a higher *original* chiasma frequency than the long-chromosome race, although they have the same frequency at metaphase; this conclusion is in agreement with theoretical considerations (PHILP and HUSKINS **13**).

9. The present observations when compared with earlier ones indicate that the three following conditions immediately determine the degree of terminalization and therefore the form of the bivalents in non-hybrid organisms: (1) the length of the two chromosomes (for the chiasmata of fragments in *Fritillaria* are always terminal, while the long chromosomes have little terminalization); (2) the number

and position of the chiasmata; (3) the rate of movement of the chiasmata in different positions and in different relationships with one another. With regard to the last condition, it is evident that the difference between *Tulipa*, *Pisum*, and *Rosa* on the one hand and forms like *Primula*, *Campanula*, and *Oenothera* with complete terminalization on the other, lies in the *rate* of movement, not in its duration, for the movement continues in *Tulipa* until metaphase. The difference between *Fritillaria* and *Tulipa* seems also to consist in rate of movement, for although the change (so far as it goes) seems usually to have ceased in *Fritillaria* (as seen in fragments) at the earliest observed stage, and the number of chiasmata is not later reduced, the terminalization coefficient is found to be higher at metaphase than at diplotene.

10. Since changes in position occur with little or no change of number and at a stage before records are possible (v. par. 7), it follows that BELLING's observations of internode length at diakinesis do not necessarily bear on the original spatial relationship of the chiasmata at the earliest diplotene; the apparent interference might be determined by a tendency for the equalization of the size of loops. Numerical evidence (cf. HALDANE 9) is not invalidated, however, since little numerical change occurs in the earliest stages of terminalization or throughout all stages in *Lilium*, *Fritillaria*, and *Vicia*.

11. The high proportion of terminal chiasmata observed in *Triticum* hybrids with few chiasmata (DARLINGTON 3) is in strong contrast with the observations in these non-hybrid forms, and suggests that the conditions of zygotene pairing in these hybrids are different from those in non-hybrids, probably owing to polarization of the nucleus and interruptions of the linear sequence (DARLINGTON 6).

12. The observations on terminalization in hybrid roses (ERLANSON 7) differ from the present in three respects: (1) the terminalization coefficients are higher; (2) the number of chiasmata in the rose "Orleans" is considerably reduced between diplotene and diakinesis although the proportion of terminal chiasmata is low; and (3) the number of chiasmata is often reduced from a higher number to one. The third result is perhaps associated with the first, but the second requires a special explanation, which is perhaps as follows: if two chiasmata are arrested in terminalization by a change of homology

and they compensate for each other, they will cancel each other without reaching the end of the chromosome. The observation of arrest of single chiasmata by change of homology in this form of *Rosa*, which was shown in this and in other ways to be a structural hybrid, makes this conclusion probable.

Summary

Observations on meiosis in *Tulipa* show that it has incomplete terminalization of chiasmata between diplotene and metaphase. They support the theory of behavior earlier adopted, especially in indicating that the original position of a chiasma influences its chance of becoming terminal. They also make it probable that terminalization occurs in all organisms, and further investigations will be undertaken to analyze the slight changes between diplotene and metaphase that have hitherto escaped notice.

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LITERATURE CITED

1. BELLING, J., The attachments of chromosomes at the reduction division in flowering plants. Jour. Gen. **18**:177–205. 1927.
2. ———, Chiasmas in flowering plants. Univ. Calif. Publ. Bot. **16**:311–338. 1931.
3. DARLINGTON, C. D., Chromosome studies in *Fritillaria*. III. Chiasma formation and chromosome pairing in *F. imperialis*. Cytologia **2**:37–55. 1930.
4. ———, Meiosis in diploid and tetraploid *Primula sinensis*. Jour. Gen. **24**: 65–96. 1931.
5. ———, Meiosis. Biol. Rev. **6**:221–264. 1931.
6. ———, The analysis of chromosome pairing in *Triticum* hybrids. Cytologia **3**: (in press). 1931.
7. ERLANSON, EILEEN W., Chromosome organization in *Rosa*. Cytologia **2**: 256–282. 1931.
8. GAIRDNER, A. E., and DARLINGTON, C. D., Ring formation in diploid and polyploid *Campanula persicifolia*. Genetica **13**:113–150. 1931.
9. HALDANE, J. B.S., The cytological basis of genetical interference. Cytologia **3**: (in press). 1931.
10. NEWTON, W. C. F., Chromosome studies in *Tulipa* and some related genera. Jour. Linn. Soc. Bot. **47**:339–354. 1926.

11. NEWTON, W. C. F., and DARLINGTON, C. D., Meiosis in polyploids. I. Triploid and pentaploid tulips. Jour. Gen. **21**:1–16. 1929.
12. PELLEW, C., and SANSOME, E. R., Genetical and cytological studies on the relations between Asiatic and European varieties of *Pisum sativum*. Jour. Gen. (in press). 1931.
13. PHILP, J., and HUSKINS, C. L., The cytology of *Matthiola incana* R. Br., especially in relation to inheritance of double flowers. Jour. Gen. **24**:359–404. 1931.
14. SAX, K., Chromosome structure and the mechanism of crossing over. Jour. Arnold Arboretum **11**:193–220. 1930.
15. ———, The mechanism of crossing-over. Science **74**:41–42. 1931.

SIGNIFICANCE OF CHROMOSOME BEHAVIOR DURING DIAKINESIS IN OENOTHERA

J. THERON ILLICK

(WITH SIXTEEN FIGURES)

Introduction

The material of *Oenothera* upon which part of this study is based was collected during the summers of 1928 and 1929 from the experimental garden of Professor G. H. SHULL, Princeton, New Jersey. The 1928 collection consisted of the following:

O. angustifolia (mut. from *lamarckiana* according to DEVRIES), pedigree no. 2746, plant no. 28,

O. bullata, pure, pedigree no. 2750, plant no. 3,

O. mut. from *lamarckiana*, pedigree no. 2768, plant no. 4,

O. lamarckiana sulfurea, pedigree no. 27216, plant no. 17.

Several collections were made in each instance from 10 to 12 o'clock in the morning, and fixed in the field. Both P.F.A.$_{15}$ and P.F.A.$_3$ were used, giving equally good results. Both fixing fluids were made up fresh each day collections were made.

The 1929 collection was as follows:

O. stenophylla, selfed, pedigree no. 2863, plant no. 2,

O. stenophylla, selfed, pedigree no. 2866, plants no. 6 and 10,

O. ammophila, selfed, pedigree no. 2873, plant no. 46,

O. biennis cruciata, pedigree no. 2876, plant no. 41,

O. aurata×*bullata*, pedigree no. 28114, plant no. 43,

O. aurata×*bullata*, pedigree no. 28133, plant no. 21b,

O. vetaurea supplena, selfed, pedigree no. 28254, plant no. 62,

O. (*rc. V. o.*), pedigree no. 28293, plants no. 9 and 62,

O. seg. *aurata*, pedigree no. 28356, plants no. 33 and 61.

In this instance also, several collections were made during the latter half of the morning and the earlier part of the afternoon at the height of the flowering season. They were fixed in the field in P.F.A.$_3$ which was made up fresh each day. The time for the fixation of this collection was from two to three days, after which they were washed

in 70 per cent alcohol, and in 85 per cent alcohol were taken to Nanking, China, for study. The material from both the 1928 and 1929 collections was imbedded in paraffin, cut 8 μ thick, and stained in Haidenhain's iron-alum haematoxylin.

A brief review of the principal events only will be recounted here for all stages in the development of the pollen mother cells except that of diakinesis. For a fuller account, with figures of corresponding stages, see ILLICK (11). Figures of diakinesis only are shown in the present paper, together with a full descriptive account.

Early heterotypic prophase

From the archesporial stages to the opening of the second contraction, that is, synizesis, nothing need be added to what has already been described for the pollen mother cells of *Oenothera* species so far studied cytologically (CLELAND 1, 2, 3, 4; ILLICK 11, and others). The spireme, apparently, is one continuous threadlike structure which becomes more pronounced and thickened and often beaded in appearance immediately preceding and following the first contraction mass. The nucleolus, resembling a chromatin reservoir, appears to give out its substance into the linin fiber of the spireme thread. During this period of synizesis, before and after the first contraction, the mass of reticular threads shows no significant parallelism or splitting of any kind.

The open spireme stage, which is relatively short in duration, arises immediately upon the loosening of the first contraction mass. It is at this time that the spireme clearly exhibits its continuous structure, which is relatively uniform in thickness, greatly shortened, and with only a very few fine threads present.

Immediately following the open spireme stage there sets in the second contraction. It is during this contraction period that the continuous spireme becomes segmented or constricted into the characteristic number of somatic chromosomes. This indicates a telosynaptic rather than a parasynaptic arrangement of the chromosome pairs. From this second contraction mass project loops of the thickened and shortened spireme which vary in size and number. The sides of these loops may represent members of the homologous chromosome pairs. Twisting with apparent fusion of the sides of

these loops is common, this probably representing the best opportunity during the whole prophase period for the mechanical process of crossing over.

Late heterotypic prophase

It is during this period that the chromosome configurations, so characteristic of *Oenothera* species, are to be seen. Since this diakinetic stage is of particular interest in any attempt to explain the peculiar end-to-end arrangement of the chromosomes in circles, special attention has been paid to it and figures of only this stage are given.

Table I gives the pedigree and plant numbers, the chromosomal configurations, and the genetical constitution of the eleven forms here described.

Heterotypic metaphase, anaphase, telophase, and interkinesis

The most outstanding behavior of the chromosomes during the metaphase and anaphase stages is the characteristic zigzag formation of the chromosome circles (CLELAND **3**, ILLICK **11**, etc.). Each chromosome, whether as a member of a circle or of a free pair, usually becomes attached at its mid-point to a group of spindle fibers. The chromosomes often become V-shaped, with their apices pointing toward the poles for which they are destined. The alternate chromosomes of a circle usually are seen to be moving to the same pole. Such a mechanism tends to assure an equal numerical distribution of the chromosomes of a circle to their respective poles. Here also similarity in appearance of the chromosomes makes it impossible to distinguish maternal from paternal elements, and it is assumed that homologous chromosomes lie adjacent to each other but arranged in an indeterminate fashion (SHULL **14**, ILLICK **11**).

The movement of the chromosomes to their respective poles is rapid. During telophase and throughout interkinesis the individuality of the chromosomes is retained. The interkinetic period is a long one, during which time the nuclei and their chromosomes grow in size. The latter have split longitudinally and the members of these respective pairs become curved and arrange themselves back to back, assuming a Maltese cross appearance. The nucleolus reappears *de novo*, rapidly increasing in size.

Homoeotypic mitosis

During the approach of this period, following interkinesis, the chromosomes become small and dumb-bell shaped, and lie scattered

TABLE I

PEDIGREE AND PLANT NOS.	CHROMOSOME CONFIGURATIONS	GENETICAL CONSTITUTION, ETC.
O. angustifolia, 2746(28)	Circle of 12 and 1 pair (fig. 1)	Gene mutant from lamarckiana by DEVRIES; angustifolia×stenophylla gives lamarckiana
O. bullata, pure, 2750(3)	Circle of 12 and 1 pair (fig. 2)	Gene mutant from lamarckiana by SHULL
O. mut. from lamarckiana, 2768(4)	Circle of 12 and 1 pair (fig. 3)	Gene mutant by SHULL
O. lamarckiana sulfurea, 27216(17)	Circle of 12 and 1 pair (fig. 4)	Hybrid derivative from lamarckiana×biennis
O. stenophylla, selfed, 2863(2)	Circle of 12 and 1 pair (fig. 5) (also shows circle of 6 and 4 pairs as more common configuration)	Mutant from lamarckiana by DEVRIES; stenophylla×angustifolia gives lamarckiana
O. stenophylla, selfed, 2866(6) (10)	Circle of 6 and 4 pairs (figs. 6, 7)	cf. preceding
O. ammophila, selfed, 2873(46)	Circle of 12 and 1 pair (fig. 8) (also shows circle of 14 as less common configuration, SHEFFIELD)	Described by FOCH, 1904, from German sand-dunes where it had been introduced from America (GATES, 1930)
O. biennis cruciata, 2876(41)	Circles of 8 and 6 linked together (fig. 9)	Mutant from biennis by STOMPS; differs from wild biennis in having narrow petals; biennis shows circle of 14 or circles of 8 and 6, the latter more common
O. aurata×bullata, 28114(43)	Circle of 6 and 4 free pairs (fig. 10)	aurata shows circle of 4 and 5 pairs; bullata shows circle of 12 and 1 pair
O. aurata×bullata, 28133(21b)	Circle of 6 and 4 pairs (fig. 11)	cf. preceding, also aurata and bullata
O. vetaurea supplena, selfed, 28254(62)	Circle of 12 and 1 free pair (fig. 12)	"old gold" and "double flower" from lamarckiana
O. (rc.V.o.), 28293(9) (62)	Circle of 12 and 1 pair (figs. 13, 14)	Expected to segregate for "outside in"
O. seg. aurata, 28356(33) (61)	Circle of 4 and 5 pairs (figs. 15, 16)	Segregate from franciscana (7 pairs or circle of 4 and 5 pairs)×biennis (circle of 14 or circles of 6 and 8); in other words an F_2 from a cross between vetaurea×sulfur (SHULL); breeds true; in its ancestry it has lamarckiana

upon the bipolar spindle. The chromosomes soon become regularly arranged upon the equator of the spindle, following which an equa-

tional division results. Thus four telophasic nuclei are formed from each pollen mother cell, giving rise to four granddaughter cells or microspores.

Discussion

In the light of the forms here described, as well as of all other *Oenothera* species so far studied, I wish to consider briefly in this discussion the principal hypotheses that have been suggested to explain the significance of the chromosome configurations during diakinesis.

The different hypotheses that have been advanced by CLELAND (**2, 4, 5, 6, 7**) and adopted by others in connection with the peculiar chromosome linkages in *Oenothera* are: (1) that the chromosome configuration for any species, mutant, etc., is constant and characteristic for such individuals; (2) that the number of linkage groups in any species, mutant, etc., corresponds to the number of closed circles and pairs of chromosomes present in such individuals, and a variation in the size and number of such closed circles or pairs respectively indicates a change in the number of linkage groups, with a consequent change in the genetical behavior of the plant; (3) that the arrangement of the chromosomes in closed circles is determinate; (4) that the end-to-end arrangement of the chromosomes is due to their heterozygosity; and (5) that segmental interchange is possibly an agent in the production of heterozygosity with a consequent formation of closed circles.

In connection with point 1, that the chromosomal configuration for any mutant, species, etc., is constant and characteristic for such individuals, it should be recalled that the writer (**11**) previously enumerated eight different genotypes (two of which were crosses), each of which showed more than one chromosomal configuration.

In a recent compilation of all available cytological studies so far made on *Oenothera* by different investigators, there was found a total of 28 species, mutants, half-mutants, segregates, and hybrid derivatives which have shown variable chromosomal configurations. (Six of the eight previously mentioned are included in these 28; crosses are not included, although many of these, as might be expected, have also shown variables.) In some instances there were as many as three different configurations for one phenotype. The

configurations represented 7 types of variations out of a possible 15 for the 14 chromosomes. In none of these cases was there recorded any corresponding genetical variation which might be associated with the observed cytological differences.

Two heterozygotes should also be mentioned. In a bud of the triple heterozygote, *O. pervirens*, selfed, 27132 (41), not included with these 28, there were found two configurations in two cells of the same bud, four sections apart. The one cell showed a closed circle of 14 and the other a closed circle of 12 and 1 pair.[1] Again, in the heterozygous plant, 28322(39*c*), of a *lamarckiana* type, there were found in adjoining cells of the same loculus two different configurations, a closed circle of 12 and 1 pair and a closed circle of 14.[1]

Apparently chromosomal configurations are only relatively constant features of very many of the species of *Oenothera* so far studied, and to this extent the idea that configurations are constant for any particular genotype is not justified.

In regard to the idea suggested in point 2, that closed circles represent linkage groups and that changes in the chromosomal configurations indicate corresponding changes in the number of these groups, there is evidence available which makes doubtful any such hypothesis. Some of this evidence is to be seen in connection with the facts given in the preceding paragraphs, in which in no instance (where variable chromosomal configurations appeared in any species, etc.) were there known to be any genotypical changes corresponding to these different chromosomal configurations. This would indicate that the idea that closed circles represent linkage groups is doubtful, at least for the species, etc., concerned. HOEPPENER and RENNER (10), in an extensive cytological and genetical study of eight different *Oenothera* species and their behavior in crosses, feel that, inasmuch as the flower-size factor behaves independently of the presence or absence of large closed circles, the idea that chromosome cohesion is at the basis of extensive linkage in *Oenothera* should be viewed with reserve. More cogent evidence may be expected from testing the hypothesis of SHULL (14), later adopted by ILLICK (11), that the chromosomes of closed circles are more probably indeterminate in arrangement. This idea

[1] Figures not published.

is also contrary to the hypothesis suggested under point 3, which holds to a determinate arrangement of the chromosomes. To test this hypothesis, SHULL several years ago began making appropriate crosses involving factors of the three known linkage groups. A simultaneous segregation of such factors in selfed triple heterozygotes whose chromosomal configurations continue to show completely closed circles of 14 or not more than two closed circles, or a closed circle of 12 and 1 pair of chromosomes, would indicate an indeterminate arrangement of the chromosomes in closed circles, with independent assortment, and would definitely refute the idea that closed circles represent linkage groups. The evidence so far available involves the triple heterozygotes 27127(16)(19), 27132(30)(41), 27136(5), and 27155(44), all of which are triple heterozygous *pervirens*, selfed. In each instance the chromosomal configuration is a closed circle of 12 and 1 pair, except 27132(41), which shows a closed circle of 14 in the same bud and four sections removed from the cell showing a closed circle of 12 and 1 pair. The genetical data[2] indicate, moreover, a simultaneous segregation of the members of the three known linkage groups and agree entirely with SHULL'S hypothesis for an indeterminate arrangement of the chromosomes. But since so few plants were involved in each case, the genetical data available may still be considered inadequate evidence, in this instance, for an indeterminate arrangement. Although the evidence is meager and not so strong as one would desire, nevertheless it points entirely toward an indeterminate chromosome arrangement as well as toward the idea that closed circles do not represent linkage groups.

Another case must be mentioned in this connection. *O. recidiva*, a mutant of *pratincola*, has a chromosomal configuration of a completely closed circle of all 14 chromosomes (KULKARNI 12). This is the only configuration so far observed. According to the hypothesis, *recidiva* should breed true or at least throw only two forms. When selfed, *recidiva* gives two viable types. A third type, it is claimed, should be present but does not appear because of lethal factors. Of the two viable types, one resembles *recidiva* in appearance and possesses a closed circle of 14, the other is a dwarf sterile plant and shows 7 complete chromosome pairs. This is the first case so far

[2] Not published.

known of an *Oenothera* showing such segregation at the time the chromosomal configuration was definitely known to consist of a closed circle of 14. It would appear that an end-to-end union of all the 14 chromosomes to form one large closed circle does not guarantee true breeding behavior, and therefore the chromosomes probably had in this instance an indeterminate arrangement.

Point 4 assumes heterozygosity to be the causal factor in the end-to-end arrangement of the chromosomes characteristic of so many species of *Oenothera*. Evidence has been accumulating which is contrary to such an assumption. Recently GATES and SHEFFIELD (8) presented a case of a triple heterozygote (*biennis*×*rubricalyx*) ×*ammophila*, which, according to the assumption just mentioned, should show at least one closed circle of several chromosomes. Their cytological examination of the pollen mother cells, on the other hand, showed complete pairing of the 14 chromosomes. The parents of this cross have shown the following configurations: *ammophila*, a closed circle of 12 and 1 pair (fig. 8) or a closed circle of 14; *biennis*, a closed circle of 14 or closed circles of 6 and 8; and *rubricalyx*, a closed circle of 6 and 4 pairs or a variable condition. Since this is a triple hybrid showing 7 complete pairs of chromosomes, they feel that this "makes it clear that complete pairing cannot be regarded as a sign of the homozygous condition." The question arises why such a heterozygous individual does not show closed circles as would be expected according to the suggested hypothesis.

Another similar case of a triple heterozygote (ILLICK, to be published), which was especially made up by Professor SHULL, is *f.S.o.* ×*lam.V.oi.* with pedigree 28367(230), a non-*lamarckiana* type, showed 7 complete pairs. There are 6 other heterozygotes which have been studied, whose configurations also consist of a complete pairing of the 14 chromosomes: (seg. *decipiens*×*grandiflora*)F_2, (*grandiflora*×seg. *decipiens*)F_2, mut. *sulfurea*×*grandiflora*, *grandiflora*×mut. *sulfurea*, (*lamarckiana*×*rubricalyx*, Afterglow)F_1 selfed, and another pedigree of (*lamarckiana*×*rubricalyx*, Afterglow)F_1 selfed. According to the assumption, such heterozygous individuals should have some of their chromosomes joined together in closed circles.

In addition to the heterozygotes prepared through appropriate

crosses, the following examples should be given in support of this contention: *O. franciscana sulfurea*, a hybrid derivative of *franciscana*×*biennis*, has thus far shown two extreme configurations, 7 complete pairs, and a closed circle of 12 and 1 pair, and yet is recognized as being very stable genetically, as is *franciscana* which is claimed to be a pure species (CLELAND). If *franciscana sulfurea* is homozygous, how can the closed circle of 12 and 1 pair be explained on the assumption that closed circles result from heterozygosity? In this hybrid derivative, moreover, no genetical changes have been observed which correspond to the observed changes in its chromosomal configurations. *O. grandiflora* B has shown three extreme configurations, a closed circle of 14, a closed circle of 4 and 5 pairs, and 7 complete pairs in different individual plants. *Grandiflora* B also is known to be very stable in its breeding behavior, and no genetical changes have been observed that correspond with the changes in its chromosomal configurations. It is difficult to understand how heterozygosity can be the cause of the large-circled forms of *grandiflora* B and at the same time have individuals showing the 7 paired condition. *Grandiflora* Ait. (GERHARD 9) also has shown three configurations, a closed circle of 14, a closed circle of 12 and 1 pair, and 7 complete pairs. GERHARD claims that this species is a "complex heterozygote" and does not breed true, but splits off twin hybrids in each generation, one-third of which are *ochracea*, a 7 paired segregate. Here we have the difficulty of explaining a 7 paired condition in an individual known to be a "complex heterozygote," as well as the difficulty of explaining segregation in an individual with a closed circle of 14 chromosomes on the basis of the assumptions just mentioned.

In addition to these three forms, which show complete pairing as well as configurations consisting of large closed circles, there are four others, *pervirens*, *rubrinervis*, *flava*, and *franciscana*, which present the same problem as those already considered. Each of these has shown two or more configurations of 7 complete pairs and large closed circles. In each case there is doubt brought to bear upon the question of heterozygosity as the factor involved in the formation of closed circles.

Of a total of 15 species of *Oenothera*, therefore, none of which is a

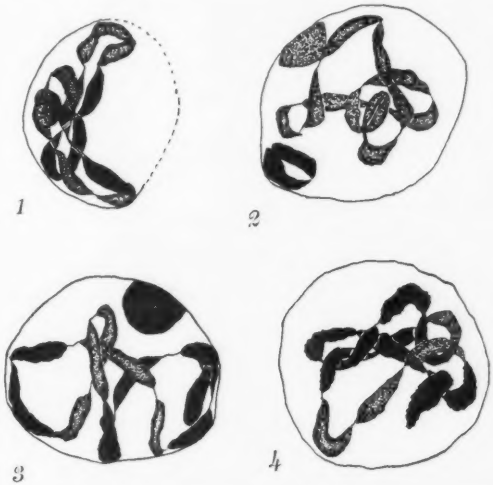
cross, that have shown at some time complete pairing, 8 have shown complete pairing only, while the other 7 in addition to the 7 pairs have shown one or two configurations consisting of closed circles. In none of these cases, moreover, were there recognized any genetical changes that corresponded to the changes in the chromosomal configurations.

The question naturally arises, if heterozygosity is not the causal factor involved in the formation of closed circles of chromosomes, what is the cause? It was first suggested by SHULL and later by the writer (**11**), and independently by SHEFFIELD (**13**), that such catenation of the chromosomes in *Oenothera* may be due to a gene or a combination of genes as other genotypical characteristics are determined.

Out of 25 triple heterozygotes (ILLICK, to be published) especially made up by SHULL, 17 have shown a chromosomal configuration which corresponds to one or both parents of the cross. Of the 8 others, the configuration of one parent in each cross was unknown, while the configuration shown by the F_1 was unlike the configuration of the known parent. Further data on the chromosomal configurations of these 8 unknown parents might later reveal a similarity between the F_1 and one or both of these parents in respect to this characteristic. A similarity in chromosomal configuration between one or two parents and the F_1 of these 17 triple heterozygotes indicates an inherited characteristic which might well be expressed as due to gene action. Of 53 other heterozygotes whose configurations are known, 14 showed configurations like both parents and 17 showed configurations like one parent, when both parents are known, while 19 showed configurations unlike both parents and 3 were unlike one parent when the other parent was unknown. In other words, the chromosomal configurations of 31 heterozygotes might be said to be an inherited trait. In the case of the 19 heterozygotes whose configurations were unlike both parents and the 3 heterozygotes whose configurations were unlike the one parent known, further examination might reveal configurations resembling one or both parents.

Other instances can be cited as follows, in favor of the idea of genes as an explanation of closed circles. *O. stenophylla*, selfed, 2863

(2) and 2866(6)(10), figures 5, 6, and 7, shows two configurations, a more common condition of a closed circle of 6 and 4 pairs, and a closed circle of 12 and 1 pair. *O. stenophylla* is a mutant from *la-*

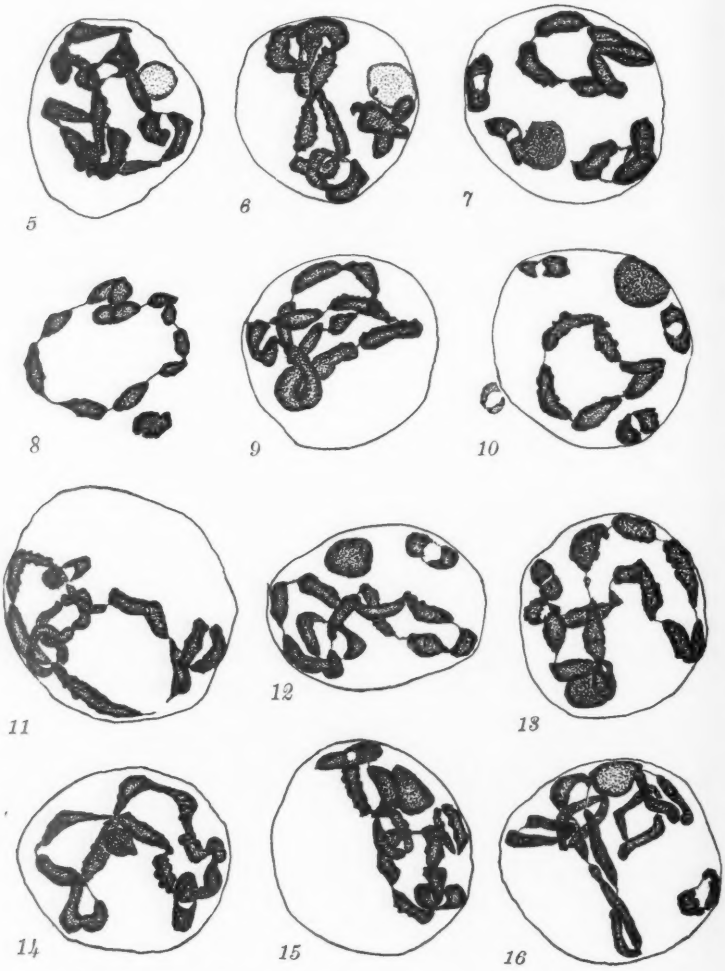


FIGS. 1–4.*—Fig. 1, *Oenothera angustifolia* (mut. from *lamarckiana* according to DEVRIES), pedigree no. 2746 and plant no. 28: Approaching late prophase with chromosomes shortening and nuclear membrane disappearing; a closed circle of 12 chromosomes is present together with small nucleolus; the free pair of chromosomes not present. Fig. 2, *O. bullata*, pure, pedigree no. 2750 and plant no. 3: Approaching late prophase with chromosomes in closed circle of 12 chromosomes well differentiated; one free pair present; nucleolus heavily stained, lying close to nuclear membrane. Fig. 3, *O.* mut. *lamarckiana*, pedigree no. 2768 and plant no. 4: Approaching late prophase with closed circle of 12 chromosomes and 1 chromosome of the free pair; nucleolus large, darkly stained, and lying close to nuclear membrane. Fig. 4, *O. lamarckiana sulfurea*, pedigree no. 27216 and plant no. 17: Approaching late prophase with closed circle of 12 chromosomes and 1 chromosome of the free pair; nucleolus not present. ×2700.

* Figures 1–4 made with Bausch and Lomb microscope with binocular eyepiece attachment, oculars 7.5, and oil immersion 1.3. Nuclear elements were drawn at their different levels and shaded accordingly. Collection made in 1928.

marckiana (deVries) as is *angustifolia* (deVries). When these are crossed they give *lamarckiana*. In each instance of parent and offspring, a closed circle of 12 and 1 pair has been observed.

O. biennis cruciata, 2876(41), figure 9, is a mutant from *biennis* by STOMPS, and shows the configuration of closed circles of 6 and 8



FIGS. 5–16.*—Fig. 5, *O. stenophylla*, selfed, pedigree no. 2863 and plant no. 2: Approaching late prophase with closed circle of 12 chromosomes and 1 pair which is linked to circle; nucleolus is small and failed to stain darkly except around periphery. Figs. 6, 7, *O. stenophylla*, selfed, pedigree no. 2866 and plant nos. 6 and 10: Approaching late prophase; in each plant (figs. 6 and 7 respectively) there is a closed circle of 6 chromo-

* Figures 5–16 made with Bausch and Lomb microscope with binocular eyepiece attachment, ocular 7.5, and oil immersion 1.9. Collection made in 1929.

which also is seen in *biennis*. It is similar to *biennis* genetically except for narrow leaves.

O. seg. *aurata*, 28356(33)(61), figures 15 and 16, is a segregate from *franciscana*×*biennis*, breeds true, and has in its ancestry *lamarckiana*. The configuration observed is a closed circle of 4 and 5 pairs as has also been seen in *franciscana*.

O. angustifolia, 2746(28), figure 1; *O. bullata*, pure, 2750(3), figure 2; *O.* mut. from *lamarckiana*, 2768(4), figure 3; and *O. lamarckiana sulfurea*, 27216(17), figure 4, a derivative from *lamarckiana*×*biennis* —these all are either mutants or derivatives of *lamarckiana* and so far have shown only one type of configuration, a closed circle of 12 and 1 pair as is also the common configuration of *lamarckiana*.

Each of these instances offers evidence in support of the idea that chromosomal configurations are inherited traits and as such the result of gene action.

In regard to the assumption that segmental interchange plays a part in determining the production of closed circles, the evidence is still insufficient. The question might be raised why the chromosomes of *Drosophila*, where segmental interchange has been recognized and studied, have not formed closed circles. It would seem that other factors are involved in the production of closed circles. From the

somes and 4 pairs; in plant no. 6 (fig. 6) of the 4 pairs, 2 are free and 2 are linked to the circle; nucleolus is still large but fails to stain darkly except around periphery; endonucleolus present; in plant no. 10 (fig. 7) the 4 pairs are free and nucleolus is darkly stained. Fig. 8, *O. ammophila*, selfed, pedigree no. 2873 and plant no. 46: Late prophase, nuclear membrane absent and chromosomes much shortened; closed circle of 12 chromosomes and 1 free pair; nucleolus not present. Fig. 9, *O. biennis cruciata*, pedigree no. 2876 and plant no. 41: Approaching late prophase; two closed circles of 8 and 6 respectively which are linked together; nucleolus absent. Fig. 10, *O. aurata*×*bullata*, pedigree no. 28114 and plant no. 43: Approaching late prophase with closed circle of 6 and 4 free pairs (one of the free pairs lies outside the nuclear membrane, possibly carried out by the knife); nucleolus heavily stained. Fig. 11, *O. aurata*×*bullata*, pedigree no. 28133 and plant no. 21*b*: Approaching late prophase; closed circle of 6 chromosomes and 4 pairs; one pair free, one pair linked to circle, and the other two pairs linked to circle and to each other; nucleolus absent. Fig. 12, *O. vetaurea supplena*, selfed, pedigree no. 28254 and plant no. 62: Approaching late prophase with closed circle of 12 chromosomes and 1 free pair; nucleolus heavily stained. Figs. 13, 14, *O.* (*rc.V.o.*) will segregate for "outside in." Pedigree no. 28203 and plant nos. 9 and 62 respectively: Approaching late prophase with closed circle of 12 chromosomes and 1 pair in both instances; in plant 9 (fig. 13) the pair is free, while in plant 62 (fig. 14) the pair is attached to circle; nucleoli of both cells heavily stained although much smaller in fig. 14. Figs. 15, 16, *O.* seg. *aurata*, pedigree no. 28356 and plant nos. 33 and 61 respectively: Approaching late prophase with closed circles of 4 chromosomes and 5 pairs in both instances; in plant 33 (fig. 15) three pairs are free and the other two are linked to circle, while in plant 61 (fig. 16) three pairs are free and the other two are linked together. ×3300.

evidence here presented it would be more reasonable to assume gene action as the causal factor.

Summary and conclusions

1. A brief review is given of the principal stages in meiosis for eleven mutants, hybrid derivatives, segregates, and hybrids, together with a more detailed account of diakinesis with figures.

2. Additional evidence to that given in 1929 is presented, based upon all available cytological studies so far made on *Oenothera*, to show that chromosomal configurations at diakinesis are only relatively constant features of the genus.

3. Since in no instance, where the chromosomal configurations were found to be variable, were there observed any genotypical changes corresponding to these variable configurations, it is doubtful whether closed circles represent linkage groups.

4. Evidence is given of the simultaneous segregation of factors representing the three linkage groups in triple heterozygotes of appropriate crosses as well as segregation in a form showing a closed circle of 14 chromosomes, which points toward an indeterminate arrangement of the chromosomes in closed circles.

5. Considerable evidence is presented which throws doubt upon the assumption that closed circles result from heterozygosity.

6. The evidence given supports rather the idea that chromosomal configurations are inherited traits and as such are the result of gene action or interaction.

7. The evidence in favor of segmental interchange as a factor to explain the end-to-end arrangement of the chromosomes is still considered insufficient.

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LITERATURE CITED

1. CLELAND, RALPH E., The reduction divisions in the pollen mother cells of *Oenothera franciscana*. Amer. Jour. Bot. **9**:391–413. 1922.
2. ———, Meiosis in pollen mother cells of *Oenothera franciscana sulfurea*. BOT. GAZ. **77**:149–170. 1924.

3. ———, Chromosome behavior during meiosis in the pollen mother cells of certain Oenotheras. Amer. Nat. **59**:475–479. 1925.

4. ———, Meiosis in the pollen mother cells of *Oenothera biennis* and *Oenothera biennis sulfurea*. Genetics **11**:127–162. 1926.

5. ———, The genetics of *Oenothera* in relation to chromosome behavior, with special reference to certain hybrids. Verhandl. Internat. Kongr. Vererbungswissensch. Berlin. 1927. 554–567. 1928.

6. ———, Meiosis in the pollen mother cells of the Oenotheras, and its probable bearing upon certain genetical problems. Proc. 4th Internat. Bot. Congr., Ithaca. 1926. **1**:317–331. 1929.

7. ———, Chromosome behavior in the pollen mother cells of several strains of *Oenothera lamarckiana*. Zeitschr. Indukt. Abstam. Vererb. **51**:126–145. 1929.

8. GATES, R. R., and SHEFFIELD, F. M. L., Chromosome linkage in certain *Oenothera* hybrids. Phil. Trans. Roy. Soc. London B. **217**:367–394. 1929.

9. GERHARD, KARL, Genetische und zytologische Untersuchungen an *Oenothera grandiflora* Ait. Jenaische Zeitschr. Naturwiss. **64**:283–338. 1929. (Original article not seen.)

10. HOEPPENER, E., and RENNER, OTTO, Genetische und zytologische Oenotherenstudien. II. Zur Kenntnis von *Oenothera rubrinervis, deserens, Lamarckiana-gigas, biennis-gigas, franciscana, Hookeri, suaveolens, lutescens*. Bot. Abhandl. **15**:1929. (Original article not seen.)

11. ILLICK, J. T., A cytological study of meiosis in the pollen mother cells of some Oenotheras. Genetics **14**:591–633. 1929.

12. KULKARNI, C. G., Meiosis in pollen mother cells of strains of *Oenothera pratincola* Bartlett. BOT. GAZ. **87**:218–259. 1929.

13. SHEFFIELD, F. M. L., Chromosome linkage in *Oenothera*, with special reference to some F_1 hybrids. Proc. Roy. Soc. London B. **105**:207–230. 1929.

14. SHULL, G. H., *Oenothera* cytology in relation to genetics. Amer. Nat. **62**:97–114. 1928.

ANNUAL RINGS OF THUJA OCCIDENTALIS IN RELATION TO CLIMATIC CONDITIONS AND MOVEMENT OF SAND

FLORENCE WOLFE

(WITH SIX FIGURES)

Introduction

It is characteristic of sand dunes that many dead trees may be found there, particularly in regions where there are great amounts of moving sand. The appearance of these trees tends to raise questions as to when they lived and under what conditions they were covered by the sand and then uncovered again. As one sees the sand slowly sifting over into a living forest he is impressed by the great length of time that it must require for an extensive area to be destroyed.

The writer spent the summer of 1930 near one of the outstanding dune areas on the east shore of Lake Michigan, and became greatly interested in the question of the effect of moving sand upon trees growing in dune areas. Having studied the work of DOUGLASS[1] on the relation between annual rings and climatic conditions, it was thought that this method might be applied in the study of trees on the dunes.

Briefly, DOUGLASS found, by comparing the width of the rings with the past rainfall reports over a considerable period of time, that there was a closé correlation between the amount of yearly growth of the tree and the amount of rainfall for the corresponding year. Since climatic conditions seemed to indicate so directly the amount of growth, comparison was then made between different trees living at the same period of time. Comparative graphs were made showing the yearly growths of adjacent trees, and close correlation was found in many cases. Graphs from rings of dead trees were then prepared and studied in connection with those of the living trees. It was dis-

[1] DOUGLASS, A. E., Weather cycles in growth by big trees. Monthly Weather Rev. June, 1919.

covered in this case that the graphs showed an overlapping. Thus if one tree lived from 1730 to 1930 and another from 1630 to 1830, the graphs of these trees showed an overlapping of 100 years during the period of 1730 to 1830. Without a definite knowledge of the period in which a certain tree lived, graphs made of the rings and compared with those of a living tree in the same locality would show the period in which the dead tree existed. Upon this basis DOUGLASS made many interesting studies and discoveries.

It was hoped that environmental conditions in the dunes would be sufficiently fluctuating to produce marked variations in the rings of the trees, and that these variations would make it possible to determine the age of the dead trees in any given area and the rate of movement of the sand.

Most of the trees were found to be *Thuja occidentalis*, a fact which explains the reason for their survival over so long a period of time. The wood of this species is very durable, and is especially adapted to withstand extremes of heat and cold when in contact with the sand. A few remnants of other trees were found, but these were in such a poor state of preservation that little information could be obtained from a study of them; consequently, the entire investigation deals with *T. occidentalis*.

Region studied

The region studied is located at the west end of Crystal Lake in Benzie County, Michigan. This region has been described in some detail by WATERMAN.[2] The entire region is composed of moving sand and fixed dunes. Strong winds continually carry the sand from the shore inland. The direction of the wind is altered slightly by the presence of occasional fixed dunes and troughs. The small area studied in connection with this problem lies about 1.25 miles south of Point Betsie, near the lake shore. In general three situations are present: the fixed dune area, the large area of moving sand, and the group of dead trees in the moving sand. The fixed dune area is largely composed of *Thuja occidentalis*, *Ostrya virginiana*, *Tilia*

[2] WATERMAN, W. G., Development of root systems under dune conditions. BOT. GAZ. 68:22–53. 1919.

americana, and *Abies balsamea*. The long narrow stretch of fixed dunes nearest the lake is only a remnant of the conifer forest left in the midst of moving sand. This area and the one lying east of the dead trees are of the same general character. The largest specimens of *T. occidentalis* average about 15 inches in diameter and about 30 feet in height.

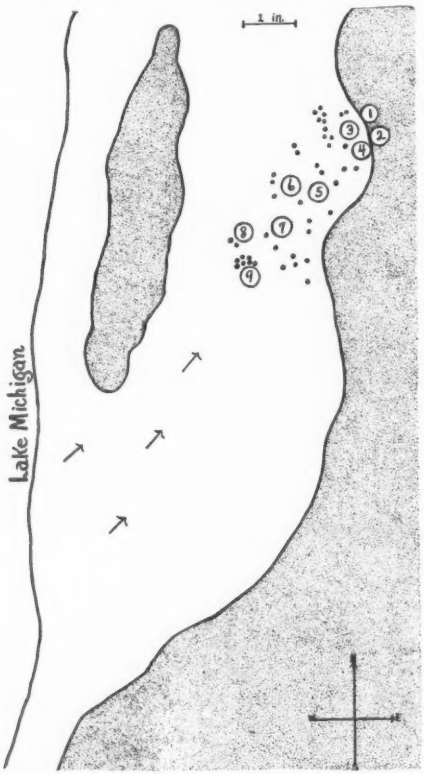


FIG. 1.—Map of region under study: fixed dunes indicated by stippling; moving sand by plain area; direction of wind by arrows; trees used in graph (fig. 6) by circles with numbers; other dead trees by large dots. Scale: 1 inch = 125 feet.

The arrows on the map (fig. 1) indicate the direction in which the wind blows most of the time. It sweeps in from the shore and carries the sand in a northeasterly direction over into the forest. This is illustrated in figure 2, which was taken looking toward the north between the relic on the left and the forest on the right.

After a careful survey of the entire area, it seemed apparent that the dead trees and those of the living forest on the east were constituents of a homogeneous living forest at some time in the past. How long ago did these trees live, and how long has it taken the sand to destroy this area, leaving only remnants of *T. occidentalis* as evidence of the devastation?

Procedure

A Swedish increment borer proved satisfactory for the removal of cores from the trees. The cores used for the graphs were in one section, extending from the outside of the tree to the center, and were about 0.37 inch in diameter. Considerable difficulty was encountered in obtaining good specimens, because in many cases the trees were weathered so much that part of the last few years of growth was lost, or the centers were soft, water-soaked, and often rotted. Some of the trees were in good condition, however, and afforded excellent material.

FIG. 2.—Detail of dead conifer forest. (Photograph by W. G. WATERMAN)

The cores were numbered and placed in boxes, being kept in place by grooves of corrugated cardboard. The size of the tree and its location were recorded. In all cases the cores were taken from the west side of the tree, in order that there would be the least possible chance for variation due to difference in position. Generally they were taken 2–3 feet above the sand level and 3–8 feet above the soil level. This variation in vertical position was not considered a serious matter, as DOUGLASS found little variation in correlation of rings at different heights in the yellow pine and sequoia. The width of the rings was measured under a hand lens by a finely marked ruler. This width was tabulated and then graphed.

Before work was begun upon trees of this area, borings were made of trees in other regions near by, in order to find whether there was sufficient variation in the rings to make cross graphing possible.

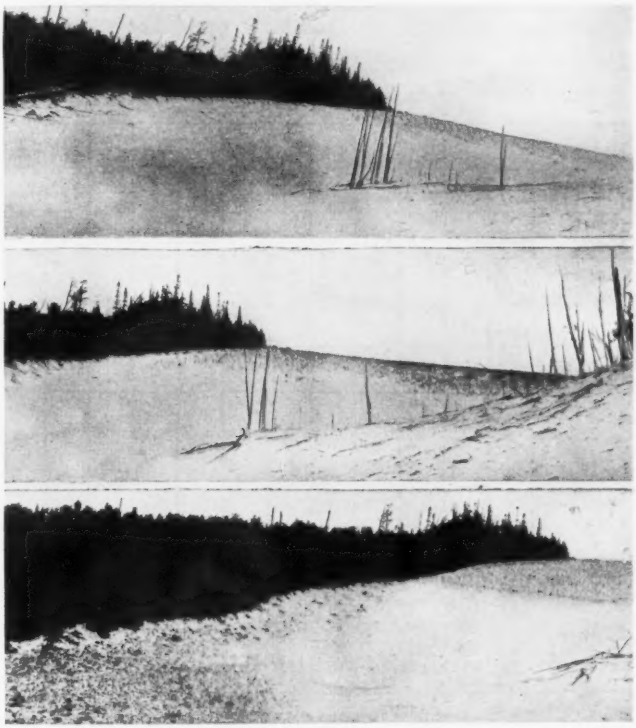
After making a great number of borings of living trees, it was found that those in a homogeneous area showed high correlation. However, trees growing in a valley and those growing on a fixed dune showed little or no correlation. This fact led to the conclusion that if the dead and living trees of this particular area showed correlation, they must belong to a group which grew under the same environmental conditions.

Borings were first made of the living trees, partially covered by the sand on the east slope of the moving dune. Others were made of trees which were dead and located near the living ones. These had been covered about one-third of their height and had died because of this covering. Still other borings were made of dead trees at different distances to the southwest of the first group, that is, in the direction from which the sand apparently had been approaching. Graphs were made from the measurements of the rings of the different trees and compared in order to determine whether or not any correlation could be distinguished and whether there was any relationship in the dates of their deaths.

It is evident that if the area was at one time a continuous living forest, the graphs should show an overlapping, but the records of the trees in the extreme southwestern part should show an earlier death than those in the northeast, since those farthest to the southwest would be covered and killed first.

This particular locality has always attracted attention on account of the rapid movement of the sand and its relation to ancient conifer patches. Photographs were taken at intervals of several years, and they (figs. 3–5) illustrate the relatively small changes brought about by the movement of the sand. These were taken from approximately the same position, figures 3 and 4 being more nearly identical. Figure 3 was made in 1912, figure 4 in 1918, and figure 5 in 1929. The first shows a central group of trees which has changed considerably in appearance by 1918 and remains as a pile of fallen trunks in 1929. It will be noted that sand has been removed from the bases of these trees and that by 1929 the sand was advancing from the left into the lowest part of the trough. On the other hand, there was practically no change in the appearance or extent of the forest patch in the background. These figures do not have any

direct bearing upon the problem itself, but may serve to emphasize the fact that a considerable amount of time is necessary for any great change to be made in an area of any size.



FIGS. 3–5.—Group of dead trees on Point Betsie dunes with relict patch of conifers in background: fig. 3 (upper view), 1912; fig. 4 (middle view), 1918; fig. 5 (lower view), 1929. (Photograph by W. G. WATERMAN.)

Discussion

In order that the meaning of the graphs be made clear it will be necessary to refer to figures 1 and 6, because the location of the tree is significant. Trees nos. 1 and 2 were young and very thrifty. They were located at the bottom of the lee slope of the approaching sand dune, being covered for 1–2 feet at the base. The close correla-

tion of their yearly growth may be noted. Trees 3 and 4 were situated well back in the bank of approaching sand, so that they were covered approximately 6 feet or more. These two trees were all dead except one small branch on each. It will be seen that the effect of the sand has been apparent during the past 20 years of the life of each of them. The same increase is seen in growth of nos. 1, 2, 3, and 4 during the years 1899 and 1900. It is evident that from the

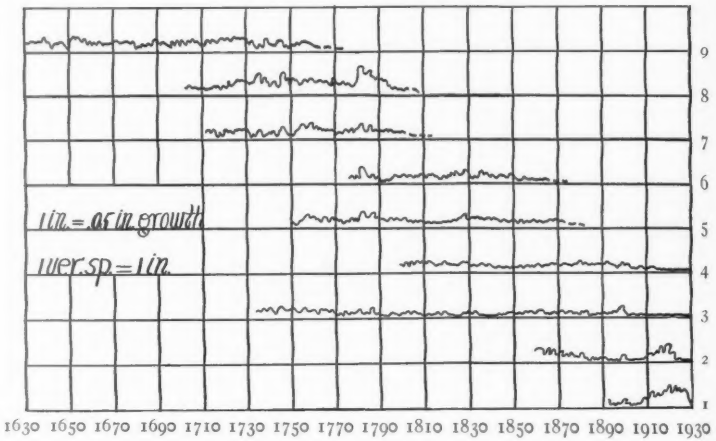


FIG. 6.—Graph showing annual growth and dates of existence of trees. Scale indicates inches: horizontal space = 10 years' time; vertical space = 1 inch or 0.05 inch of growth.

beginning environmental conditions left a definite impression on the amount of growth of all of the trees in that particular year.

Borings nos. 5 and 6 were taken from trees several feet southwest of the preceding two. They were dead, and had been so for a considerable length of time, although there were a few remnants of bark left at the base of each. The curves prepared from their rings correlate with a period much farther back in the history of tree no. 3. Their living existence was closed at approximately 1875–1880. The marked correlation of nos. 3, 4, 5, and 6 during the years 1827–1830 is obvious. Records for nos. 7 and 8 were taken from trees still farther toward the southwest, as may be seen on the map (fig. 1). They died between 1805 and 1812. There is unusual irregularity in

the curves during the period between 1780 and 1790, the same irregularity being shown in trees 5 and 6.

Boring no. 9 was taken from a tree in the extreme southwesterly portion of the dead forest, not far from the group of dead trees mentioned in the preceding section. Its history dates back to about 1630, its death occurring about 1770. There is an unusual correlation of nos. 7, 8, and 9 during the period of 1724–1730. The correlation in the remainder of the period is rather close, although there are some variations, as might be expected.

Borings of several other trees were made, some of which showed very similar conditions while some were more difficult to correlate. The borings of the ones shown in figure 6, however, are sufficient to show the situation during the past century and a half. The time elapsed since the first trees were killed by the sand until the present is nearly 160 years. The distance between the two points is 500 feet. The average forward movement of the sand has been about 3.12 feet per year during the past 160 years.

Summary

1. Climatic conditions in Michigan sand dunes are sufficiently variable to produce marked effects in the annual growth of *Thuja occidentalis*.

2. A graph showing the annual growth of each of a group of trees indicates the period of existence of the dead trees.

3. The time required for the destruction of this particular area was approximately 160 years.

4. The active sand dune in this region averaged 3.12 feet per year in its progression into the living forest. About 20 years is required for the life of a tree to be terminated by sand moving at this rate.

5. The destruction of living forests by the moving sand in this dune region occurs very slowly, as revealed by the records of the annual growth of *Thuja occidentalis*.

The writer wishes to express her gratitude to Dr. W. G. Waterman, of Northwestern University, for his kind assistance and criticisms during the progress of this work.

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BUESIA, A NEW SUBGENUS OF HYMENOPHYLLUM FROM PERU[1]

C. V. MORTON

(WITH ONE FIGURE)

Several years ago Mr. C. BÜES, of Quillabamba, Peru, collected in the Department of Cuzco a considerable number of ferns, which Professor FORTUNATO L. HERRERA, of the University of Cuzco, has since made available for study. The series of Hymenophyllaceae is especially rich, including numerous rare species as well as several which are new to science. One of the latter, representing a new subgenus, is here described.

HYMENOPHYLLUM subg. **Buesia,** subg. nov.—Filix pendula, rhizomatibus longe repentibus; stipites et rhaches paleis pluri-cellularibus basi cellulis 2- vel 3-seriatis planis instructi; rhaches primariae secundariaeque flexuosissimae; laminae elongatae pinnatae, pinnis tripinnatipartitis; segmenta ultima serrata; sori in lobulis intimis contractis abbreviatis dispositi; indusium vix immersum bifidum margine undulatum; receptaculum subglobosum crassum.

Species typica: *Hymenophyllum mirificum* Morton.

Further collections may show that the present species represents a distinct generic type. The presence of true scales on the stipes and rhachises has been unknown elsewhere in *Hymenophyllum*. The sole reference to scales in the genus is by METTENIUS,[2] who mentions their occurrence in "*H. cristatum* Wall." Since there is no such species by WALLICH, the name is probably a misprint for *H. crispatum*. GOEBEL[3] remarks on this point in describing his new genus *Hymenophyllopsis* and decides that METTENIUS is in error, the "scales" of *H. crispatum* being merely hairs. The subgenus *Buesia*, however, has small but relatively numerous true scales, linear-subulate in out-

[1] Published by permission of the Secretary of the Smithsonian Institution.

[2] METTENIUS, G., Über die Hymenophyllaceen, Abhandl. Math.-Phys. Klasse der Königl. Sächs. Gesells. Wiss. 7[2]: 467. 1864.

[3] GOEBEL, K., Archegoniatenstudien. XVIII. Flora N.S. 24[1]: 1–37. 1929.

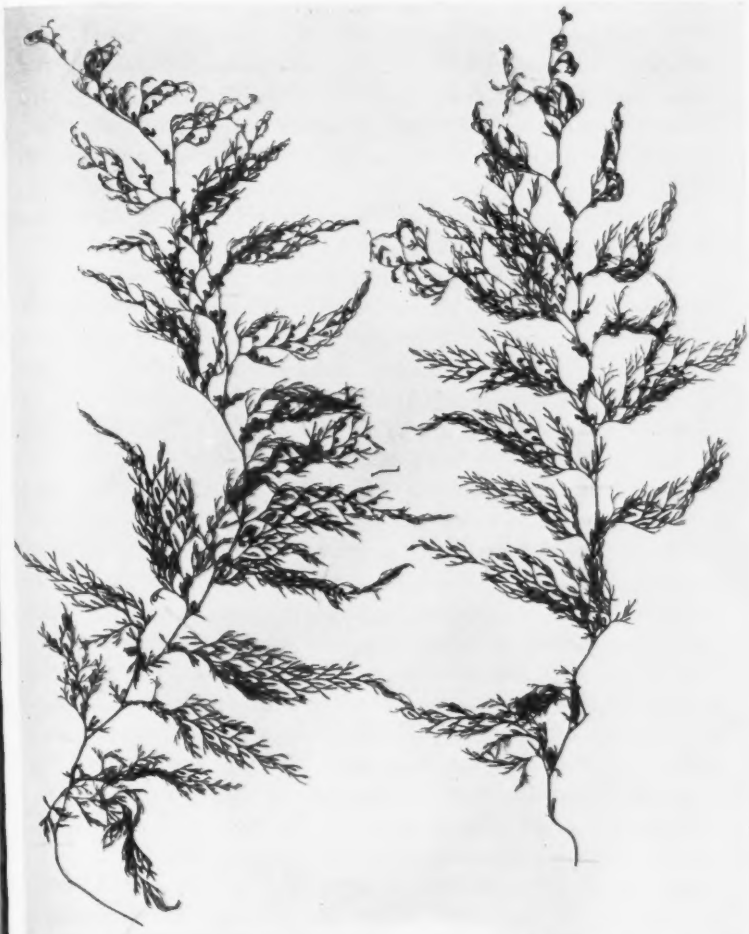

FIG. 1.—*Hymenophyllum mirificum* Morton, type specimen; approximately half natural size

line. They are two or three cells wide at the base, tapering to a single cell at the apex.

The subglobose receptacle present in *Buesia* is found in only a few species grouped together by PRANTL as *Hymenophyllum* Sect. *Globosa*, namely, *H. caudiculatum* Mart. of South America, *H. dilatatum* Sw. of New Zealand, and *H. junghuhnii* v. d. B. of Java. These three species are closely related to one another but are very different from *H. mirificum*, which by its serrate margins is obviously related to the subgenus *Leptocionium* (which, however, has a filiform receptacle, often more or less exserted in age).

The habit, too, of *Buesia* is distinctive. The extremely flexuose rhachis is striking and is unlike that of any heretofore known species of *Hymenophyllum*. The sporangia of *H. mirificum* were not in good condition for study, but despite that fact the relationship of the species to *Hymenophyllopsis* seems even more remote than to *Hymenophyllum* proper. The frond is only one cell thick and the indusia are very similar to those of *H. fucoides* Sw.

Hymenophyllum mirificum sp. nov.—Rhizoma longe ad truncos arborum repens, parce ramosum, paleis setiformibus ad nodos instructum, mox glabrescens, ca. 1 mm. diametro, fuscum, radicibus comparate numerosis, pilis flavescentibus numerosissimis juventute obsessis; stipites in rhizomate biseriati, ca. 7 cm. longi, 0.5–1 mm. crassi, teretes, fusci, paleis lineari-subulatis brunneo-hyalinis, cellulis basi 2- vel 3-seriatis, numerosis flaccidis sparsim obsiti, demum glabrati; rhachis flexuosissima teres, haud alata, 0.5–1 mm. diametro, paleis eis stipitis similibus mox deciduis parce obsita; rhaches secundariae evidenter flexuosae alatae; laminae pendulae ambitu elongato-oblongae vel raro fere lineares, ca. 30 cm. longae, 15 cm. latae, interdum minores, pinnatae, pinnis ipsis tripinnatipartitis, glabrae, planae haud undulatae; pinnae primariae lanceolatae, multijugae, spatiis 11–16 mm. longis separatae, interdum petiolulatae, maximae ca. 23 cm. longae et 2.5 cm. latae, superiores minores; segmenta secundaria lanceolata, maxima ca. 3 cm. longa, ad apicem pinnarum gradatim minora; segmenta tertiaria simplicia vel in lobulos perpaucos divisa; segmenta ultima anguste vel angustissime linearia, maxima 8 mm. longa et 0.5 mm. lata (raro pauca usque ad 1 mm. lata), marginibus perspicue arguteque serratis, dentibus

apicem segmentorum versus ca. 6 utroque latere; nervi dichotomi, nigri, apicem segmentorum non attingentes; pinnae pinnulaeque terminales, steriles; sori numerosi, subaxillares, in lobulis intimis valde abbreviatis contractisque, quam lobulis multo latiores; indusium vix in segmentis immersum, ovale, 2–2.5 mm. longum, 1.5 mm. latum, basin cuneatum versus 3/4 bifidum, apice rotundatum et irregulariter undulatum; receptaculum dilatatum, subglobosum, crassum.

Type in the U.S. National Herbarium, no. 1,515,604, collected at Raccaypata, Vilcabamba, Prov. de la Convención, Dept. Cuzco, Peru, in humid forest, alt. 3600 meters, July 8, 1921, by *C. Bües* (no. 1600).

Mr. BÜES has collected five additional numbers of this species. The collections, which are all from Prov. de la Convención, are as follows: Type locality, Jul. 8, 1921, *Bües* 1592, 1593; Sueracocha, alt. 4000 meters, Jul. 12, 1924, *Bües* 1587; Alturas de Sieve, alt. 3300 meters, June, 1924, *Bües* 1551; road from Llactapata to Michihuañuncca, Huadquiña, alt. 2800–3000 meters, October, 1920, *Bües* 711.

U. S. NATIONAL MUSEUM
WASHINGTON, D.C.

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BRIEFER ARTICLES

SOME SUBARBORESCENT SPECIES OF NICOTIANA

From 1799, when RUIZ and PAVON described *Nicotiana tomentosa*, to date, eight related species of subarborescent Nicotianas have been described: *N. wigandioides* K. Koch & Fint in 1858, *N. otophora* Griseb. in 1879, *N. colossea* André in 1888, *N. rusbyi* Britton in 1900, *N. friesii* Dammer in 1903, *N. herzogii* Dammer in 1916, and *N. leguiana* Macbride in 1930. Except in the case of *N. tomentosa*, the authors of certain of the species more recently erected have, apparently, been unaware of the existence of the other related species which had been described at earlier dates. For example, in describing *N. rusbyi*, BRITTON makes no reference to *N. wigandioides;* and in describing *N. herzogii*, DAMMER apparently was not aware that *N. rusbyi* and *N. wigandioides* had been described, nor, in defining *N. friesii*, does he indicate knowledge of the existence of *N. otophora*.

Recently it has been possible to compare types or co-types of all the eight species just mentioned, with the possible exception of *N. colossea*. On the basis of this evidence it is clear that *N. wigandioides*, *N. rusbyi*, and *N. herzogii* are indistinguishable morphologically; that *N. otophora* and *N. friesii* are equivalent; and that it is doubtful whether anything is gained by attempting to distinguish *N. colossea* and *N. leguiana* from *N. tomentosa*. On the basis of priority, therefore, the species of the broad genetic group in question are considered to be *N. tomentosa* R. & P., *N. otophora* Griseb., and *N. wigandioides* K. Koch & Fint. It now appears necessary to add a fourth species, *N. tomentosiformis*, which as the proposed designation implies is definitely related to *N. tomentosa*. All except *N. otophora* are grown in the Botanical Garden of the University of California.

In preparation are detailed discussions of the cytogenetic relations, morphology, and taxonomy of all the nine named species, which will include proper description of *N. tomentosiformis* in its relationship to the other three valid members of the group. It may here be said that the species under discussion possess in common a subarborescent habit, a more or less marked zygomorphic tendency in the flower, and a flower which shows a tendency to develop anthocyanin coloration. They have

a range of distribution from central Peru to southern Bolivia and northern Argentina, at altitudes of from 700 to 3000 meters. As a group they exhibit certain morphological relationships to *N. glutinosa* L. and *N. tabacum* L.

This preliminary reference to the taxonomic situation and to the proposal to describe the new species *N. tomentosiformis* is necessitated primarily by the fact that in current cytogenetic literature the designation *N. rusbyi* is given to a form quite distinct from the species described under this name by BRITTON, a name which, as already indicated, is to be considered a synonym of *N. wigandioides*. In 1921 Dr. O. E. WHITE, while a member of the Mulford Biological Expedition engaged in exploration of the Amazon basin, collected seed of a treelike shrub which was very common in some of the mountain valleys of the Bolivian Yungas between Quime and Canamina. At the time these plants were identified as *N. rusbyi*, and under this name plants from this seed have been grown in this Botanical Garden and elsewhere, and with them a number of cytogenetic investigations are in progress.

In order to eliminate the confusion in nomenclature now obtaining, and particularly because it is definitely distinguishable from the other three valid species to which it is related, a separate designation for what has been grown as *N. rusbyi* appears justified. The type of *N. tomentosiformis* is "Mulford Biol. Expl. of the Amazon Basin, #75; coll. *H. H. Rusby;* vic. Canamina, Bolivia; alt. 4000 ft.; July 15, 1921."—T. H. GOODSPEED, *University of California.*

MIOCENE INSECT GALL IMPRESSIONS

(WITH ONE FIGURE)

An interesting discovery was made while studying a collection of fossil leaf impressions from Late Miocene[1] shales in Douglas Canyon, situated in the N.W. quarter of the S.W. quarter of Section 30, T. 23 N., R. 24 E., Douglas County, Washington.

A single, imperfectly preserved leaf specimen, *Quercus cognatus*, was found to contain 25 gall-like impressions scattered over its surface (fig. 1). These gall-like impressions, circular in outline, ranged in diameter from 0.3 cm. to 0.4 cm., and were characterized by a dotlike protuberance at their centers.

[1] HOFFMAN, A. D., The Douglas Canyon flora of east-central Washington. Jour. Geology 1932 (to be published).

Omitting from consideration the element of color, illustrations of these impressions closely resembled the illustrations not only of the Cynipid

FIG. 1

galls, *Dryophanta discus*[2] and *Neuroterus umbilicatus*,[3] but also of the Itonid gall, *Cecidomyia poculum*.[4]—ARNOLD D. HOFFMAN, *University of Chicago.*

[2] [3] [4] FELT, E. P., Key to American insect galls. N.Y. State Mus. Bull. no. 200. pp. 73, 107, 107. 1917.

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CURRENT LITERATURE

BOOK REVIEWS

Colloid chemistry

The third volume of *Colloid chemistry*, edited by ALEXANDER,[1] is devoted to technological applications of colloid chemistry. It contains 42 papers, many of which will be interesting to biologists, even though the discussions mainly concern other fields. The editor classifies 11 of the papers as dealing with general principles, 6 with more specialized mechanical problems, and 25 telluric problems (that is, dealing with things of the earth). These latter papers are on geology and mineralogy, metals, petroleum, asphalt, and agriculture.

A few titles are given to show the diversity of material presented: some practical results of X-ray researches on colloids; surface and catalysis; colloid factors in water supply; the supercentrifuge in industry; porcelain and allied ceramic bodies; colloid systems in metallography; colloid chemistry of petroleum; deflocculated graphite.

The last four papers, which are agricultural, will be of greater or more direct interest to biologists, since three of them deal with soil problems and one with cereal chemistry. The three soil papers are: soil colloids, by DI GLERIA and ZUCKER; the colloidal chemistry of the soil, by BRADFIELD; and rapid colloidal and mechanical analysis of soils, by BOUYOUCOS and MCCOOL. The last paper is by GORTNER, on the colloid chemistry of wheat, wheat flour, and wheat flour products.

The editor of these volumes is entitled to the satisfaction he feels in having been able to secure the co-operation of so many able investigators in elucidating the manifold aspects of colloidal physics and chemistry. The tasks of authors, editor, and publishers have been well performed, and the result is an admirable group of valuable books.—C. A. SHULL.

Shorter course in organic chemistry

A new textbook by COLBERT[2] has features that should appeal to students of chemical physiology. It has properly placed the emphasis upon the theoretical foundations of organic chemistry, and on the aliphatic compounds. The

[1] ALEXANDER, JEROME, Colloid chemistry. vol. III. pp. 655. Chemical Catalog Co., New York. 1931. $10.50.

[2] COLBERT, J. C., A shorter course in organic chemistry. 8vo. pp. xviii+352. The Century Co. New York. 1931.

aromatic compounds occupy about one-third of the space, and the heterocyclic compounds only a dozen pages in the last chapter. This is probably much less discussion of the heterocyclic compounds than most students of biology would like, since they are found so frequently in biological material. Nevertheless the emphasis is in the right direction, and the author has been content to sacrifice details for the sake of principles. He is evidently acquainted with the needs of students. Helpful reviews, stimulating questions, and problems requiring thought and calculation are properly distributed through the work at the end of chapters.

There are a number of useful charts which summarize the fundamental reactions, as of glucose, the chart of which is inserted in the chapter on carbohydrates. Another chart summarizes the kinds and the uses of cellulose; another, the chemical relationships existing among the aliphatic compounds.

The reviewer considers it a very helpful work. It should make it easier for the student who has had somewhat limited opportunities in the organic field to familiarize himself with the fundamental theories and conceptions which underlie our attempts to summarize the chemistry of the life processes of organisms. The author deserves much credit for the sound scientific and pedagogical principles which have gone into the work.—C. A. SHULL.

Spring flowers

A paper-covered book[3] with carefully condensed contents has just been published that is likely to prove most useful to the student who wishes to become familiar with the vernal flora of the Great Lakes region. It abounds in keys for the larger and smaller groups, while the drawings of the plates are so well condensed that about 200 species are sketched in a recognizable manner. It would have made the book more useful if it had been made up in smaller size, so that it could be carried conveniently in the pocket.—G. D. FULLER.

MINOR NOTICE

Sixth International Botanical Congress

According to a decision by the Fifth International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive Committee has been formed, the President of which is Professor F. A. F. C. WENT (Utrecht), while Professor J. C. SCHOUTE (Groningen) will act as Vice-President, Dr. W. C. DE LEEUW (Bilthoven) as Treasurer, and Dr. M. J. SIRKS (Wageningen) as Secretary. The Sixth Congress will meet at Amsterdam, September 9–14, 1935. Scientific societies are kindly requested to remember these dates in planning their own meetings.

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[3] ROSENDAHL, C. O., and FREDERIC, K. B., A guide to the spring flowers of Minnesota. pp. xi+89. *Pls. 25*. University of Minnesota Press. 1931. $1.00.